Chapter 5
Biological Resources

By
R.F. Van Dolah, A.F. Holland, L.D. Coen, A.H. Ringwood, M.V. Levisen, P.P. Maier, J.D. Jones, B. Anderson, Y. Bobo, D. Richardson, G.I. Scott, A.K. Leight, M.H. Fulton, J.W. Daugomah, P.L. Pennington, and D.E. Chestnut

Introduction:

A major concern identified by the Clean Water Task Force was to protect biological resources in the estuarine waters of Beaufort County (Clean Water Task Force, 1997). This issue was highlighted by the closure of several hundred acres of shellfish harvesting grounds in 1995, combined with the knowledge that more than 46,000 acres of estuarine habitat in Beaufort County were already closed to this type of activity. Task Force members were also concerned that other estuarine biota may be experiencing problems due to increased pollution and other anthropogenic stresses related to the increased coastal development in the county.

Due to these concerns, a major objective of this study was to evaluate the condition of key biological resources inhabiting several different types of habitats in both Broad Creek and the Okatee River. Our study focused on sampling shellfish and other bottom-dwelling biota that have proven to be good indicators of environmental stress in other studies (Pearson and Rosenberg, 1978; Scott et al., 1992; Dauer, 1993; Fulton et al., 1997; Weisberg et al., 1997; Coen and Luckenbach, 1999; HEED report, 1998; Van Dolah et al., in press). We did not attempt to survey the condition of all the aquatic biota, including the fish and crustacean species that are recreationally or commercially important. Many of these species are transient, which may not be indicative of localized conditions, or they are difficult to capture in sufficient densities that would allow us to adequately assess the condition of those populations in the two drainage systems. Specific biota that were sampled included the following taxa:

1. Macrobenthic communities inhabiting the bottom sediments of both tidal creek and open-water areas of each drainage system,

2. Intertidal oyster (*Crassostrea virginica*) populations at six locations along the length of each drainage system,

3. Grass shrimp (*Palaemonetes pugio*) populations inhabiting the tidal creek and shallow open-water habitats of both systems, and

4. Mummichog (*Fundulus heteroclitus*) fish populations inhabiting the tidal creek habitats of each system.

A description of the study objectives related to each of these biotic resources is provided in the following sections, along with a summary of the methods and results obtained.

Benthic Macrofauna*:*

The benthic macroinvertebrate communities inhabiting the bottom sediments of Broad Creek and the Okatee River represent a very important biological resource of concern. These organisms include a diversity of worms, crustaceans, mollusks, and other taxa that live in tubes or actively burrow through the sediments. The benthos are an extremely important component of the estuarine and marine food web since they are consumed by a large number of predatory species, including most of the fish and larger crustaceans (e.g. shrimp and crabs) that are harvested for recreational or commercial purposes.

Due to their relatively sessile life habits and their high exposure to the sediments, the benthos also have been documented to be excellent indicators of habitat condition (Pearson and Rosenberg, 1978; Dauer, 1993; Weisberg et al., 1997). Studies in southeastern estuaries have clearly documented that the condition of benthic assemblages is correlated to contaminant exposure and/or degraded bottom water quality, in both open-water habitats and the tidal creeks that drain upland areas (Hyland et al., 1996, 1998; Holland et al., 1996; Lerberg, 1997; Sanger, 1998). As part of a regional assessment of estuarine habitat condition, studies of the benthos have resulted in the development of an Index of Biological Integrity (IBI). This IBI showed a much greater efficiency of correctly classifying sediments which were chemically degraded when compared to various laboratory bioassays that were conducted using the same sediments (Hyland et al., 1998; Van Dolah et al., 1999).

Specific objectives of the benthic macrofaunal assessment were to:

1. Evaluate the composition and condition of macrobenthic assemblages in shallow tidal creek habitats located along the length of each drainage basin,

2. Evaluate the condition and composition of macrobenthic assemblages inhabiting both intertidal and subtidal sediments of open-water habitats located along the length of each drainage basin, and

3. Compare the condition of the macrobenthic assemblages sampled in Broad Creek and Okatee River with studies that have assessed the condition of macrobenthos in other estuarine drainage systems in South Carolina.

Methods:

Samples of the macrobenthos and associated sediments were collected at 15 sites in each drainage basin during August, 1997. Six sites were located in tidal creeks, six

were in subtidal mainstem river areas of the larger drainage system, and three were located on intertidal flats along the shoreline of the mainstem river portion of each drainage system (Figures 5.1 and 5.2). The tidal creek and subtidal river sites were randomly located within each of the six subzones established along the length of each drainage system (see Chapter 2). The intertidal flats were also randomly selected from the three larger zones representing the upper (headwater), middle and lower (seaward) portions of each drainage system. All station positions were located using differentially-corrected Geographic Positioning Systems (GPS).

Sampling in each of the tidal creeks was restricted to the upper (landward) 300 m section and followed procedures similar to those described by Holland et al. (1996) and Lerberg (1997). Ten cores (7.6 cm diameter x 15 cm depth) were collected at random locations along the length of the creek section approximately one meter below the mean high water (MHW) level to assess the composition of the benthic community. All cores were collected from non-vegetated sediments. Each core was washed separately through a 0.5 mm sieve and the benthic fauna retained on the sieve were preserved in a 10% buffered formalin-seawater solution for later identification in the laboratory.

Ten smaller core samples (3.5 cm diameter x 15 cm depth) were collected adjacent to the benthic cores for analysis of sediment composition (% sand, silt-clay). Another sample of the surficial sediments (top 3-5 cm) was also obtained adjacent to each benthic core using a stainless spoon for analysis of sediment contaminants, sediment toxicity, acid volatile sulfides (AVS), and pore-water ammonia (See Chapter 4). These latter samples were composited into one sample for each sampling site.

Benthic infaunal assemblages at the subtidal and intertidal stations within the mainstem portion of each drainage system were sampled using procedures similar to those described by Hyland et al. (1998). Six to eight grab samples were collected from an anchored boat using a 0.04 m^2 Young grab, with the boat repositioned between each sample. Three of the samples (generally grabs 1, 3 and 5) were washed through a 0.5 mm sieve to collect the benthic fauna, which were preserved and processed as described above. Prior to sieving each sample, a small core (3.5 cm diameter) was inserted into the grab sample to collect sediments for analysis of grain size (% sand, silt-clay). The remaining grabs were used to collect sediments for analysis of contaminants, sediment toxicity, AVS and pore-water ammonia concentrations. Only the top 3-5 cm of sediments were collected from these grab samples using a stainless steel spoon to form a single composite sample for each station (see Chapter 4 for additional information). Samples from the three intertidal stations were collected at a depth of approximately 1 m below MHW during the period of high tide. Depth varied at the subtidal stations dependent on their location.

All benthic data were analyzed using a variety of parametric and non-parametric statistics to compare the various biological measures considered among drainage systems as well as among stations within each drainage system. Most of these analyses were completed using either one-way or two-way analysis of variance tests (ANOVA) on either the raw data or transformed data if required to meet test criteria. Post hoc

comparisons were generally completed using either the Tukey or Bonferonni test (Sigmastat, 1994). When transformations did not correct the data, ANOVAs were completed on ranked values using the Kruskal Wallis test. Statistical comparisons completed between two groups utililized either the t-test or Mann-Whitney U test if assumptions for the parametric test were not met.

Findings:

Subtidal River Stations:

Faunal Abundance:

The overall average abundance of benthic organisms collected at the six subtidal stations in Broad Creek was significantly lower when compared with the Okatee River stations ($p < 0.002$). This was primarily due to the large differences observed among sites at stations R-3, R-4 and R-5 (Figure 5.3). The greatest differences were observed at station R-5 in Broad Creek, where faunal densities were much lower than observed at all other stations sampled in both drainage basins. In contrast, station R-5 of the Okatee River had an unusually high faunal density compared to all of the other sites, primarily due to a very high density of one amphipod species (*Ampelisca vadorum*, Table 5.1).

Even though we observed significant differences among stations in the two drainage systems, most of the stations in both systems had densities similar to those observed at relatively pristine sites with comparable physical conditions in Port Royal Sound and Mackay Creek (Van Dolah et al., 1991; Wendt et al., 1991). Faunal densities in Broad Creek and the Okatee River were also similar to those observed at other "undegraded" stations sampled elsewhere in South Carolina during the summer of 1995 by the Environmental Monitoring and Assessment Program (Hyland et al., 1998).

Subtidal river stations located in the lower portions of each drainage system tended to have higher faunal densities than the stations in the headwater areas (Figure 5.3). For example, animal densities at stations R-1 and R-2 (upper zones) in the Okatee River were significantly lower than densities observed at the other four stations. Similarly, stations R-4 and R-6 in Broad Creek had significantly higher faunal densities than stations R-1 to R-3 (upper half) of that drainage system.

Faunal densities were not correlated with sediment composition (% sand, silt-clay) or average salinities observed at the sites. Both of these variables are known to influence infaunal densities, but there was not a large difference among the sites sampled with respect to these physical/chemical characteristics (see Chapter 4). The relatively reduced faunal densities observed in the upper portions of each drainage system may have been related to greater variances in salinities that are likely to routinely occur at these sites compared to the lower stations. A large variance in salinity causes increased stress in many estuarine species, especially those that are not tolerant of low salinity conditions that would likely occur after major rain events. Our hydrographic sampling

did not include a long enough period of record to adequately assess the range of salinity variance that may occur at the stations sampled.

Faunal Diversity:

A statistical comparison of the average number of species collected at the subtidal river stations indicated that the Broad Creek had significantly fewer species per site than the Okatee River when all stations were considered collectively ($p < 0.002$). However, this was primarily due to differences observed at stations R-4 and R-5 (Figure 5.3). Comparisons made within zones indicated that the number of species were similar in both rivers at stations R-1 to R-3 and significantly greater at station R-6 in Broad Creek.

Hyland et al. (1998) provide evidence that stations having fewer than three species/0.04 m^2 grab are often degraded based on other environmental measures (e.g. high sediment contaminants, low dissolved oxygen, or significant sediment toxicity). Only station R-5 in Broad Creek had fewer than three species/grab, and all of the other sites had a substantially greater number of species (average of 10-42 species/grab; Table 5.1). These species richness values are comparable to those observed at a relatively pristine site located in Mackay Creek (Wendt et al., 1991) and at "undegraded" sites in other estuaries of South Carolina, where an average of 8-25 species were collected per grab sample (Hyland et al., 1998).

Estimates of species diversity using the Shannon Weaver H' Index also supports the hypothesis that species diversity was comparable among the two drainage systems, except at station R-5 (Figure 5.3, Table 5.1). The low H' value observed at station R-5 in Broad Creek was most likely due to the very low faunal abundance at this site. In contrast, the low H' value observed at station R-5 of the Okatee River was primarily due to the overwhelming dominance of the amphipod *Ampelisca vadorum*, which reduced the species evenness component at this site.

Hyland et al. (1998) observed that diversity (H') values below 1.0 were indicative of degraded benthic assemblages based on other measures of environmental condition. Only station R-5 in Broad Creek had H' values less than 1 and most of the other sites in both drainage systems had H' values ≥ 2.5 (Table 5.1, Figure 5.3). These values are comparable to stations that were classified as non-degraded or marginal during the EMAP surveys of southeastern estuaries in 1994 and 1995. Neither the H' values or the mean number of species collected per site were correlated with sediment composition or salinity conditions. There also was no clear gradient in the number of species collected per site versus distance from the headwater area of each drainage system. Stations in upper third of Broad Creek (R-1, R-2) had a lower species richness than we observed at stations R-3, R-4 and R-6, but the differences were only significant between stations R-1 and R-2 versus R-6 ($p < 0.05$). In the Okatee River, station R-1 was significantly lower than all other zones ($p < 0.05$), which were not statistically different from each other.

Faunal Composition:

A complete listing of benthic macrofauna collected at each subtidal station is provided in Appendix 5.1. Polychaete worms were the numerically dominant taxa in

both the Okatee River and Broad Creek (Figure 5.4, 5.5). The most abundant polychaete species *(Streblospio benedicti*) was found at all 12 river sites (Table 5.1). This species has a very ubiquitous distribution throughout South Carolina estuaries and is often found in highest abundances at sites with a mixture of sand and mud (Van Dolah et al., 1979, 1984, 1990; Lerberg, 1997). Many studies have identified this species as pollution tolerant (Pearson and Rosenberg, 1978; Hyland et al., 1985; Sanger, 1998). The relative density of this species tended to be higher at the Broad Creek sites compared to the Okatee River.

Oligochaete worms were also relatively abundant at four of the stations in the Okatee River and two of the stations in Broad Creek (Figure 5.4, 5.5, Table 5.1). The dominant species was *Tubificuoides wasselli* and unidentified specimens of Tubificidae and Enchytraeidae. The sensitivity of these taxa to pollution stresses is unknown.

At two stations (R-5 in both rivers), amphipods were the most abundant taxa. In the Okatee River, the dominant amphipod was *Ampelisca vadorum*, which was found in unusually high abundance. Little has been documented on the pollution sensitivity of *A. vadorum*, but other ampeliscid species have been shown to be pollution sensitive (ASTM, 1993; Fulton et al., 1997). In Broad Creek, the dominant species was *Protohaustorius bousfeldii*. The pollution sensitivity of this species is also unknown, but amphipods are generally considered to be pollution sensitive organisms (Swartz et al., 1984; ASTM, 1993). However, it should be noted that even though *P. bousfeldii* was the dominant species at this site, the abundance of this species was very low compared to densities of the dominant taxa at other stations in either drainage system.

An evaluation of overall species composition among the sites sampled using a similarity coefficient analysis (Bray Curtis coefficient; Bloom, 1992) indicated several interesting similarities and differences among the sites. Three major station groups were identified based on their degree of overall faunal similarity (Figure 5.6) One group consisted of the three upper stations in Broad Creek (R-1 to R-3) and the uppermost station in the Okatee River (R-1). These results suggest that faunal composition in the headwater areas of the two drainage systems are similar and that faunal complex extends further down Broad Creek than in the Okatee. This may reflect effects from greater freshwater runoff that are typically associated with urbanized systems compared to non-urbanized systems.

The two stations near the mouth of Broad Creek (R-4, R-6) were also very similar to the station nearest the seaward limit of the Okatee River (R-6) and all of these stations were relatively dissimilar to the uppermost stations. This is most probably a reflection of differences in the station salinities and salinity variance since we would anticipate conditions to be more constant and indicative of high salinity habitats in the lower reaches of each system compared to the upper reaches. Stations in the middle portion of the Okatee River (R-2 to R-5) were dissimilar to the comparable sites in Broad Creek with respect to overall similarity. However, with the exception of the relatively depauperate fauna at R-5 in Broad Creek, these differences do not appear to be indicative of pollution stress, *per se*.

Benthic Index of Biological Integrity:

A Benthic Index of Biological Integrity (B-IBI) has recently been developed for use in estuaries of the southeastern U.S. as a measure of biotic condition (Hyland et al., 1998; Van Dolah et al., 1999). This index, which includes several measures of benthic integrity, has proven to accurately classify most sites that are chemically degraded. Another benthic IBI has recently been developed specifically for South Carolina waters. This index uses a very similar approach, but a slightly different combination of benthic metrics (Van Dolah et al., unpublished). Both indices were applied to the benthic community data collected from subtidal sites in this study to determine how the stations ranked (Figure 5.7). The results indicate that only station R-5 in Broad Creek had a benthic assemblage that considered to be moderately degraded using either index. Stations R-1, located at the headwaters of each drainage system, had lower B-IBI scores than the other stations. This may reflect some increase in stress or it may be due to the fact that these stations were very shallow. The benthic index was developed using stations that were generally deeper than these sites, and may not be as applicable at the headwater sites. This was also the case for the SC B-IBI, which did not show as consistent a differential in values between the headwater stations and others located lower in each drainage system. Based on the results of both indices, the majority of stations in both drainage areas did not show evidence of biological degradation.

Intertidal River Stations

Faunal Abundance and Diversity:

The intertidal mud flat stations in both drainage systems generally had a lower abundance and diversity of macrofauna than the subtidal stations (Figures 5.3, 5.8, Appendix 5.1). These differences were anticipated since intertidal habitats are more stressful environments to marine organisms than subtidal habitats due to periodic exposure of the sediments during low tide periods. All sites had infaunal densities above values that have been used to classify degraded sites in subtidal areas using this benthic metric (Hyland et. al., 1998). Additionally, statistical comparisons among all of the intertidal sites showed no significant differences between drainage system ($p > 0.9$) or between zones within each drainage system ($p > 0.5$).

Statistical comparisons of the mean number of species found at the intertidal sites indicated that, overall, there were significantly fewer species at the Broad Creek stations than the Okatee River stations. This difference was primarily due to a significant difference observed between the lower zone stations (I-6). Sediments at the Broad Creek I-6 site had the highest cumulative contaminant level of all the intertidal sites sampled. This may be due to the high density of docks and docked vessels located along that shoreline. Overall sediment contaminant concentrations at that site were above levels that have been demonstrated to result in degraded benthic communities in subtidal areas (Hyland et al., in press). No significant differences were observed in the number of species collected in each drainage system at stations in the upper and middle sampling

zones, but Broad Creek sites had consistently fewer species than the comparable Okatee River sites.

Faunal Composition:

The numerically dominant organisms at the intertidal mud flat stations in Broad Creek consisted of a mix of polychaete and oligochaete worms (Figure 5.9, Table 5.2, Appendix 5.1) and the snail *Illyanassa obsoleta*. These species are considered to be tolerant of stressful conditions and polluted sediments, which may account for their higher abundance in this developed watershed. In the Okatee River, the dominant species were polychaete worms (Table 5.2). The most abundant of these polychaetes (*Nereis succinea, Scoletoma tenuis, Leitoscoloposl fragilis*) are also known to be tolerant of pollutants or disturbed conditions. An evaluation of overall faunal composition at the intertidal sites also showed dissimilarity between the Okatee River and Broad sites. Both Broad Creek stations I-6 and I-4 were dominated by species known to be pollution tolerant (e.g. the polychaete *S. benedicti* and the oligochaete, *M. rubroniveous* (see next section). Additionally, stations I-4 and I-6 were the only intertidal sites in both systems that exhibited sediment toxicity in both the Microtox® and seed clam assays. This toxicity could not be attributed to high ammonia levels (see Chapter 4).

The Benthic Index of Biological Integrity (B-IBI) that was developed for subtidal habitats is not applicable to intertidal environments. Comparison of other measures of benthic condition (e.g. abundance, diversity) among the intertidal sites we sampled indicated that all three Broad Creek sites were exhibiting evidence of some benthic stress. This was especially evident at station I-6, which had a much lower diversity of species present than at the comparable sites in the Okatee River. Station I-4 also coded as marginal due to the lower number of species and H' values compared to station I-4 in the Okatee.

Tidal Creek Stations:

A detailed summary of the benthic data collected from tidal creeks is provided in Table 5.3 and Appendix 5.2. A few of the samples contained mobile taxa (e.g. fiddler crabs) and very small organisms (e.g. nematodes) that were poorly sampled by the sampling methods used. These organisms were not included in the data summaries and analyses presented in this section.

Faunal Composition and Abundance:

The benthic fauna inhabiting tidal creeks was numerically dominated by segmented annelid worms, predominately oligochaetes and polychaetes (Figure 5.10). Oligochaetes accounted for 68% of the fauna, and polychaetes accounted for 27%. Mollusks (clams and snails) and crustaceans (crabs and shrimp) accounted for less than 2% of the fauna. We identified 35 benthic taxa from the 112 samples collected (Table 5.3).

Nine taxa (four oligochaetes, four polychaetes, and an unidentified insect) comprised over 90% of the fauna (Table 5.3). About half (48%) of the taxa were found

in less than three of the 112 samples. *Monopylephorus rubroniveus*, a small oligochaete (segmented worm), was the most abundant organism in Broad Creek and the Okatee River tidal creeks. *Streblospio benedicti*, a broadly distributed polychaete (another kind of segmented worm), was the second most abundant taxa (Table 5.3).

Monopylephorus rubroniveus and *S. benedicti* are well known for their tolerance to many different kinds of natural and anthropogenic stresses. *Monopylephorus rubroniveus* is the most abundant benthic organism in polluted tidal creeks of Charleston Harbor (Lerberg 1997) and has been classified as a pollution indicative species for South Carolina tidal creeks (Lerberg and Holland, in review). In laboratory experiments, this oligochaete tolerated severe hypoxia for 9 days. *Streblospio benedicti* is also a numerically dominant benthic organisms in Charleston Harbor tidal creeks, particularly undeveloped, forested creeks (Lerberg 1997). This polychaete is tolerant to exposure to low levels of dissolved oxygen, although not as tolerant as *M. rubroniveus* (Llanso, 1991; Llanso 1992).

Mollusks and crustaceans may have been rarely collected from tidal creeks because of the relatively high pore water ammonia levels that were found in the sediments from those sites. The average sediment pore water ammonium level was 16.2 and 20.4 mg/l for creeks draining into Broad Creek and the Okatee River, respectively. Experiments on juvenile hard clams suggest that these biota experience high mortality during short-term acute assays when sediment ammonia concentrations exceed about 15 mg/l (SCDNR, unpublished). Amphipods and many other benthic species experience high mortality when sediment ammonia levels exceed about 30 mg/l. Pore water ammonium levels in other intertidal and subtidal river habitats where mollusks and crustaceans were abundant were an order of magnitude lower than pore water ammonium values in tidal creeks.

The species composition and relative abundance patterns for benthic communities in tidal creeks draining into the Broad Creek and Okatee River were similar to that reported for these critical nursery habitats in other regions of the state (Swearingen 1983; LaSalle et al., 1991; Holland et al., 1997; Lerberg 1997, Sanger 1998). The oligochaetes and polychaetes that numerically dominate these habitats are hardy and productive organisms that provide a prey for juvenile fish and crustaceans. Oligochaetes have particularly high nutritional value and caloric content (Hunter and Arthur 1978; Diaz 1980; Middleditch et al., 1979).

Faunal Diversity and Abundance:

The total number of benthic species collected from the Broad Creek and the Okatee River tidal creeks were similar, 28 and 24, respectively (Table 5.3). In both Broad Creek and the Okatee River, species diversity (H') and cumulative number of benthic taxa (a measure of species richness) tended to be lowest in creeks that experienced the largest salinity range (i.e., creeks T-1 and T-6; Table 5.4). Measures of species diversity including H', cumulative number of taxa per creek, and mean number of taxa per sample were similar between the Broad Creek and the Okatee River (Figure

5.11). The biodiversity data were also similar to the values reported for higher salinity tidal creeks in Charleston Harbor (Holland et al., 1996, Lerberg 1997).

The percent of the tidal creek benthic fauna that were rare taxa was significantly ($p<0.01$) higher in Broad Creek than in the Okatee River (Table 5.3). Many of the rare taxa that were only found in Broad Creek are organisms that prefer stable, higher salinity environments (e.g., the hard clam *Mercenaria mercenaria).* The taxa that were only found in tidal creeks draining into the Okatee River were organisms which prefer lower salinity environments and can tolerate large salinity fluctuations (the oligochaete *Tubificoides heterochaetus* and the isopod *Cyathura polita*).

The overall abundance patterns for tidal creek benthic communities draining into Broad Creek and the Okatee River were similar (Figure 5.11). Highest abundances occurred in creeks that drained headwater regions (creek T-1 in Broad Creek and creek T-1 in the Okatee River) and creeks that drained salt marshes (creeks T-4 and T-5 in Broad Creek; T-5 in the Okatee River). Lowest abundances occurred in creeks T-3 and T-6 in Broad Creek. These sites were considered to be degraded (T-3) or marginal (T-6) in sediment quality and fair (T-3) or degraded (T-6) in water quality.

Effect of Salinity On Benthic Distributions:

Salinity is a major factor affecting the kinds and abundances of benthic organisms in estuarine habitats, including tidal creeks (Carriker 1967, Holland et al., 1987, Lerberg 1997). The lower frequency of occurrence of marine species and the higher abundance of benthic species tolerant of low salinities in Okatee River creeks suggest this system had a lower average salinity and more variable salinity distributions than creeks draining into the Broad Creek. The salinity data collected as part of this study did not support this finding. The average salinity and salinity range information we collected suggested salinity distributions were similar between the two systems (Tables 3.3 and 5.4). This is not surprising. Benthic community composition and their distribution patterns are developed over the life span of the organisms composing the community (months to several years). Whereas, the salinity data only represent conditions that occurred during a short period in the summer of 1997. Long-term salinity data collected by SCDHEC, however, suggests that for most of the 1990s salinity in the Okatee River was slightly higher than that in Broad Creek.

The variability in salinity over a tidal cycle (and presumably with rain events) was much higher for tidal creeks than it was in adjacent subtidal river environments (12.4 vs 2.6 ppt). Tidal creeks have limited capacity to dilute runoff compared to the subtidal river habitats. Creeks that drained large amounts of uplands (creeks T-1 and T-6 in the Broad and Okatee systems) experienced the largest salinity fluctuations (> 15ppt over a normal tidal cycle - Table 5.4). Salinity fluctuations in these ranges would tend to result in tidal creek benthic communities dominated by oligochaetes (Lerberg and Holland, in press; Lerberg, 1997).

When watersheds are developed, the amount of the land surface that is impervious to rain, including roads, parking lots and roofs, increases in proportion to the human

population density (Nemo, 1994; Arnold and Gibbons, 1996). The rate and volume of runoff into tidal creeks is generally proportional to the amount of impervious surface in the drainage basin (Kibler et al., 1981; Brown, 1988). The greater the amount of impervious surface the more peaked the runoff and the greater the salinity variance in the tidal creeks during rainfall events and over a normal tidal cycle (Holland et al., 1997). Tidal creeks located in headwater portions of estuaries drain large portions of most coastal watersheds. Salinity distributions in these headwater creeks may fluctuate from full strength seawater to freshwater over a tidal cycle. Very few benthic organisms can tolerate such extreme salinity fluctuations.

Effects of Sediment Characteristics on Tidal Creek Benthic Distributions:

The physical nature of sediments influences the kinds and abundance of benthic organisms in tidal creek habitats (Lerberg and Holland, in press; Lerberg 1997). To evaluate the effects of sediment characteristics on tidal creek benthic distributions and relative abundance, we classified tidal creek samples based on sediment properties as follows: sandy sediments (<20% silts and clays); mixed sediments (20-80% silts and clays); and muddy sediments (>80% silts and clays). Then, we summarized the benthic and sediment data for each creek and class.

Seventy-one percent of the sandy samples occurred in Broad Creek and 81% of the muddy samples occurred in the Okatee River. Samples with mixed sediments were approximately equally distributed between Broad Creek and the Okatee River, 28 and 37 respectively. Half of the creeks consisted of a single sediment class but there were creeks having all sediment classes in both systems.

Sandy sediments were numerically dominated by polychaete worms (84%) with modest oligochaete abundances (15%). Mixed sediments were numerically dominated by oligochaetes (75%) with modest polychaete abundances (23%). Muddy sediments were composed of a mixture of taxa in approximately equal proportions (24% oligochaetes, 33% polychaetes, 14% crustaceans, and 31% insects). Sand habitats had the lowest benthic abundance of all sediment classes (976 individuals/m^2), and mixed sediments had the highest benthic abundance (6,207 individuals/m^2). Benthic abundance values in the mud sediment class were intermediate (2,941 individuals/m^2). Tidal creek animal-sediment distribution patterns in Broad Creek and the Okatee River were similar to those reported for Charleston Harbor (Holland et al., 1996; Lerberg 1997). As the degree of suburbanization development increases, the headwater portions of tidal creeks draining uplands generally shift from soft mixed or muddy sediments to scoured, firm sandy habitats (Lerberg 1997, Sanger et al., 1999 a, b). The higher levels of sand and scoured sediments found in many of the tidal creeks draining into Broad Creek compared to those draining into the Okatee River support the hypothesis that higher levels of watershed development are associated with sandier sediments. The Broad Creek watershed is substantially more developed than the Okatee River watershed.

Assessment of Tidal Creek Environmental Quality

The Tidal Creeks Study conducted by the SCDNR (Holland et al., 1996) concluded that human development of coastal watersheds, including suburbanization (human population densities of >10 individuals per acre; 1-4 units per acre) adversely affected the environmental quality of tidal creek nursery habitats. The degree and magnitude of environmental impacts were proportional to the human population density and the amount of impervious surface in the watershed. Major environmental alterations to tidal creek ecosystems that were identified included the following:

(1) Increases in the rate and volume of freshwater inflow (stress indicator: salinity range over a typical tidal cycle is > 15 ppt);

(2) Frequent exposure to hypoxia (stress indicator: DO values are below 2 mg/l > 20% of the time);

(3) Increases in sediment contaminant concentrations (stress indicator: the ERLQ is > 2 or the ERMQ is > O.5);

(4) Benthic communities that are numerically dominated by pollution indicative oligochaetes, especially *Monopylephorus rubroniveus* (stress indicator: > 70% of the benthic biota are oligochaetes or *Monopylephorus rubroniveus*);

(5) Low benthic biodiversity (stress indicator: H' values < 0.4); and

(6) Depauperate benthic abundance (stress indicator: < 250 benthic organisms/m^2).

The high relative abundance of the oligochaete *Monopylephorus rubroniveus* and low species diversity values that were found in the headwater tidal creeks (T-I) of Broad Creek and the Okatee River indicate that these creeks were degraded in both systems (Table 5.4, Figure 5.12). Anthropogenic stresses that contributed to this degradation included: large salinity ranges, exposure to stressful low dissolved oxygen level, and exposure to chemically contaminated sediments.

Creek T-5 draining into Broad Creek also had a high relative abundance of oligochaetes; 78% of the fauna was *Monopylephorus rubroniveus* (Table 5.4). This creek experienced the lowest dissolved oxygen levels of any creek sampled (<0.1 mg/l) and had biologically stressful low dissolved oxygen values about 20% of the time (Table 5.4). Creek T-5 also had the highest dissolved oxygen measured. The large range in dissolved oxygen values in creek T-5 suggests that the primary producers in this creek, probably benthic microalgae, were experiencing bloom conditions during sampling. The high daytime dissolved oxygen values and low nighttime values are symptomatic of nutrient over-enrichment and algal blooms. Other indicators of stress (e.g., salinity range, sediment contaminant concentrations) were in normal/acceptable ranges for creek T-5.

Several other tidal creeks exhibited salinity fluctuations in stressful ranges (e.g., creek T-6 in Broad creek and creeks T-4 and T-6 in the Okatee River). Creeks T-3 and T-4 in Broad Creek also experienced modest (10-15 ppt over a "normal" tidal cycle) salinity fluctuations. These creeks supported benthic communities that were similar to those in undeveloped, forested reference habitats in the Charleston Harbor area, suggesting that increases in salinity fluctuations alone were not stressful enough to adversely affect benthic resources.

Several tidal creeks in the Okatee River had sediment contaminant levels that were in ranges that may cause biological harm (creeks T-4 and T-6). These creeks, however, supported "normal" benthic communities. This contamination should be considered an early warning that tidal creek sediments are repositories for contaminants (Beeftink et al., 1982, Fletcher et al., 1994, Williams et al., 1994; Sanger et al., 1999 a, b).

General Conclusions of Benthic Community Assessment:

When all of the benthic condition measures are considered collectively, 5 of the 15 sites in Broad Creek and 2 of the 15 sites sampled in the Okatee River were classified as showing evidence of minor or major stress (Figure 5.13). Only one of these sites was located in subtidal waters (R-5 in Broad Creek). We did not observe any obvious physical or chemical conditions that would account for the biotic conditions noted at the subtidal site, and the biota present there did not show evidence of severe stress. Two of the tidal creeks showed evidence of stress in each drainage system, with the effects most probably due to poor water quality conditions (e.g. effects of salinity variance or DO stress). Two additional intertidal stations in Broad Creek (mud flats) also showed evidence of benthic stress that was probably related to pollution effects.

Other sites lower in the system that showed evidence of stress related to environmental variables were the tide creek T-5 and the intertidal mud flats I-4 and I-6 in the lower portion of Broad Creek. Degradation in Creek T-5 was attributed to exposure to "below normal" dissolved oxygen levels, and degradation of the intertidal flat benthic communities was probably due to contaminant stress. All of these sites had degraded sediment quality, with toxicity observed in multiple assays.

The environmental quality and characteristics of the remaining intertidal and subtidal sites in Broad Creek and the Okatee River were similar to conditions in undeveloped forested creek in other parts of the state, with the exception of R-5 in Broad Creek. The other sites would generally be classified as "normal" with respect to the environmental quality measured as well. Among the other sites sampled, the tide creeks T-1 and T-6 in both Broad Creek and the Okatee River exhibited salinity variation that suggested biological degradation may be occurring in these creeks. Evaluation of the benthic data supports this conclusion at the T-1 sites, but not at the T-6 sites.

Oyster Populations:

As noted previously, an evaluation of the condition of oyster beds in Broad Creek and the Okatee River was of special concern to the Task Force members. In addition to their value as a recreational and commercially harvested species, oysters (*Crassostrea virginica*) serve an important ecological role since the beds form living reef structures that support a host of other associated organisms generally not found in surrounding sand or mud habitats (Coen and Luckenbach, 1999; Coen et al., 1999a,b). Studies conducted by the SCDNR have documented that oyster reefs can support resident invertebrate densities of more than 3,100 individuals/m^2 and mobile fish and crustacean densities on oyster habitat can exceed 2,100 individuals/m^2 (Wenner et al., 1996; Coen et al., unpublished). Intertidal oyster reefs are a conspicuous habitat in South Carolina and they contribute significantly to the broader functioning of the inshore waters by improving water quality through their vast filtering ability (Coen and Luckenbach, 1999; Coen et al., 199b). These filter-feeding bivalve molluscs are also excellent candidates for monitoring habitat quality (Farrington, 1983; HEED report, 1998) since they concentrate pathogenic organisms and contaminants.

SCDNR staff has been examining size-frequency relationships, recruitment potential, and disease (MSX and Dermo) levels of native oyster populations as indicators of habitat health, along with estimates of transient and selected resident species (Coen et al., 1999a; Coen and Luchenbach, 1999). These measures appear to provide an excellent indication of habitat health. Sampling of oyster habitats at more than 30 sites around South Carolina has detected large variances among sites, with developed/degraded sites often having marked differences in numbers and size-frequency relationships of oysters, as well as the numbers of mussels (*Geukensia demissa* and *Brachidontes exustus*) associated with the intertidal oyster reef matrix. Therefore, this approach was used to evaluate the oyster beds in Broad Creek and the Okatee River using the same sampling and analysis procedures. The oyster beds were also re-assessed by the DNR Shellfish Management Section using methods that have been used throughout the state, and samples were collected to evaluate the condition of the oysters within each bed.

Specific objectives of the oyster surveys were to:

1. Evaluate the density and size-frequency relationships of the living oysters in different portions of each drainage using replicate samples as a measure of population condition,

2. Assess the incidence and intensity of diseases (MSX and Dermo) in a sub-sample of oysters collected from each site,

3. Measure tissue contaminant concentrations in a sub-sample of oysters collected from each site in order to assess the relative exposure of each bed to pollution,

4. Evaluate the physiological condition of oysters collected from each site as another measure of organism health,

5. Evaluate mussel populations associated with the oyster beds as secondary measure of habitat/water quality, and

6. Re-assess the areal extent of each oyster bed sampled using procedures developed by the DNR's Shellfish Management Section and compare the results with historically collected data.

Methods:

Oyster-Mussel Collections:

Between August 26 and September 11, 1997, 60 quantitative samples were taken at 12 randomly selected sites, with one site located in each of the six sub-zones identified along the length of both drainage systems (Figure 5.1, 5.2). A 20-meter transit line was placed along each shoreline at approximately mean sea level (level with densest oyster populations (approximately 4' below MHW). Five samples (5/site x 12) were then collected at each site by placing a $0.143m^2$ quadrat at pre-selected locations along the transit line. If no oysters were located within a pre-assigned random location, another random position was chosen to ensure that five representative oyster samples were collected from each site. All oysters and sediment were removed to a depth of ~11 cm and then placed in a bag with a label. The specific quadrat placement for each sample was adjusted to maximize the percentage of oyster shell (live and dead) cover within the quadrat.

In the laboratory, a minimum of five oysters from each of the 60 samples were removed for analysis of oyster disease (n = 25/site). These oysters were measured for shell height so that they would be included in the remaining size frequency analysis. Bag contents were then washed to remove sediment and non-molluscan biota. Using calipers, each live oyster (including spat) was measured for shell height (defined as the distance from the umbo to the outermost edge) to the nearest millimeter. All oysters were examined to eliminate dead individuals. During this process, all mussels were removed, counted, and placed in labeled sample jars for later enumeration and measurements. These species were lumped to generate combined mussel total number and biomass values.

All oyster measurements (size-frequency data) were entered into an Access database for initial analysis and plotting. Plots of mean size and total number of live oysters adjusted to a per m^2 basis were generated from the above database using Sigmaplot. Cumulative size frequency distributions (5 mm intervals) were then computed for each of the 60 samples and a 2^{nd} degree polynomial fit was applied to each cumulative frequency distribution. Line parameters (intercept, slope and curvature) for each station were recorded. Statistical (ANOVA) analyses were then performed to

evaluate differences among the two drainage systems and among stations within a system.

Oyster Disease Analysis:

A subset of the oysters collected from the 60 samples (see above) were examined for two common oyster diseases (Dermo and MSX; see Bobo et al., 1997 for review). Care was taken so that we could compare infection levels of the two diseases in the same individual to see if there was a correlation between the two.

For *Perkinsus marinus* (Dermo), cell counts were determined using Ray's technique (Ray, 1952) and examined following the procedures outlined in Bobo et al. (1997). For *Haplosporidium nelsoni* (MSX) identifications, prevalence was determined from oyster histological sections fixed in Davidson's AFA, embedded in paraffin and stained in Harris hematoxylin and eosin (Howard and Smith, 1983; Bobo et al., 1997). If the data passed both the normality and equal variance tests, parametric statistical tests were performed (ANOVAs). If the data were not normal but homogeneous, both parametric (ANOVAs) and non-parametric statistics (Kruskal-Wallis [KW] test) were performed to determine differences in *P. marinus* weighted incidence (WI) and % prevalence levels, *H. nelsoni* prevalence levels, and oyster shell heights among stations and river systems. Appropriate Multiple Comparison Tests (either Bonferroni for ANOVAs or Dunn's test for K-W test) were then used to examine means when significance was detected.

Oyster Cellular Responses:

Lysosomal Destabilization:

A neutral red assay was used to evaluate lysosomal integrity (Lowe et al., 1992; Ringwood et al., 1998a). Briefly, cellular suspensions were prepared from pieces of digestive gland tissue (20-40 mg) dissected from individual oysters, and incubated in calcium/magnesium free saline and trypsin to disaggregate the cells. An aliquot of the cell suspension was mixed with neutral red on a microscope slide, covered with a cover slip, and incubated in a humidity chamber at room temperature for 60 minutes. Digestive gland cells (6 to 12 μm in diameter) containing lysosomes were examined with a light microscope (100 X under oil immersion) to evaluate NR retention. Cells with NR retained in lysosomes were scored as stabile and those with NR leaking into the cytoplasm were scored as destabilized. A minimum of 50 cells was counted for each preparation, and the data were expressed as destabilization indices (% destabilized lysosomes per individual oyster).

Lipid Peroxidation:

The thiobarbituric acid (TBA) test was used to measure lipid peroxidation (Gutteridge and Halliwell, 1990). Digestive gland tissues were homogenized in 50 mM potassium phosphate buffer (pH 7.0) and centrifuged (14,000 rpm, 4^{o}C, 5 minutes). A subsample of the supernatant was mixed with tricholoroacetic acid containing TBA and butylated hydroxytoluene, heated at 100^{o}C for 15 min and centrifuged to remove the

precipitate. The resulting malondialdehyde (MDA) was detected at 532 nm on a spectrophotometer. Standards were prepared as described by Csallany et al. (1984), and the data were expressed as nM MDA / g wet weight.

Glutathione Concentrations:

Glutathione concentrations of individual oysters were determined by the DTNB-GSSG Reductase Recycling Assay (Anderson, 1985). This assay is a sensitive and specific enzymatic procedure that follows the rate of 5-thio-nitrobenzoic acid (TNB) formation. Digestive gland tissues were homogenized in 5% sulfosalicyclic acid (SSA), and centrifuged (14,000 rpm, 5 min, 4^{o}C). Supernatants were diluted 1:1 with 5% SSA and mixed with the NADPH buffer containing DTNB. GSSG reductase was quickly added and the rate of TNB formation was monitored at 412 nm at 30 seconds intervals for 90 seconds. GSH concentrations were estimated from a standard curve and reported as nM GSH / g wet weight.

Tissue Contaminant Concentrations:

After the randomly selected quadrats had been collected, a minimum of thirty oysters were then collected by hand for tissue analysis from the mid-intertidal portion of the endemic reefs. Attempts were made to collect oysters larger than 7 cm (~ 3 in) in length. Because of the condition of some of the beds, it was necessary to collect smaller sized oysters in larger numbers to ensure sufficient tissue volume for the analyses. Efforts were made to collect the oysters within the predefined 20 m transect where possible. In some cases it was necessary to go beyond the 20 m transect to obtain sufficient numbers of oysters.

Oysters for tissue analysis were transported in coolers to the SCDHEC Aquatic Biology Section Laboratory in Columbia, SC, for sample preparation following standard SCDHEC procedures (SCDHEC, 1999). After processing, the samples were transferred to the SCDHEC Central Laboratory for analysis of constituents listed in Table 5.5. All analyses were conducted following standard SCDHEC procedures (SCDHEC, 1981; 1994).

Wet weight tissue values for detectable metals were compared to statistics derived from other oyster tissue collected by SCDHEC since 1980. Dry weight tissue concentrations were estimated by multiplying the wet weight concentration by 9.986 based on analyses completed by the SCDNR (Ringwood, unpublished). These estimates were compared with the mean value computed from national annual means from 1986 through 1993 reported by O'Connor (1996) from data collected in the NOAA National Status and Trends Program (NS&T).

Evaluation of Changes in Bed Acreage Over Time:

DNR utilized its oyster survey protocol, initiated in the early 1980s to evaluate changes in the study area over time. This resource assessment program was structured to estimate the entire State's intertidal oyster standing crop and cartographically depict the extent of its resource. Each oyster population was also characterized with respect to spatial and morphological features (e.g. percent live, cluster dispersion, volumetric variances, etc., Elliott, 1971). A similar assessment protocol was used during the Broad Creek/Okatee River study to determine changes in oyster bed size and spatial dispersion. Each oyster population was revisited, measured to determine the area covered and characterized by strata. The more conservative volume change is indicated under "live volume," while "total volume" represents the entire habitat, inclusive of dead shell and substrate. Contemporary survey data were then compared to the original in the DNR's oyster survey Geographic Information System (GIS) database.

Findings:

DHEC Shellfish Harvesting Designations:

Because shellfish harvesting pressures may influence conditions observed at the various oyster beds sampled in both drainage systems, it is important to note DHEC's classification of those sites at the time of sampling. Since the original 1997 sampling, some of the beds have changed status. The classifications at the time of the study consisted of *open* beds, where direct harvesting permitted, and *restricted* beds, where harvesting is allowed if the oysters are relayed to other locations and depurated. The status of each site in 1997 was as follows:

Broad Creek	Okatee River
O-1 Restricted	O-1 Restricted, culture permit
O-2 Restricted	O-2 Restricted
O-3 Prohibited	O-3 Open, culture lease
O-4 Open	O-4 Open, culture lease
O-5 Open, culture permit	O-5 Open, culture lease
O-6 Open, culture permit	O-6 Open, culture lease

Oyster Population Measures:

As noted above, it is important to understand that oyster densities and size-frequency distributions can be affected by past harvesting history, environmental conditions, available substrates for settlement, and recruitment of new oysters to the beds. SCDNR staff has been collecting size-frequency information throughout the state to assess and evaluate the status and trends of South Carolina's oyster resources. These data provide useful reference data for evaluation of bed conditions in the study area compared

to other parts of the state. Since sampling occurred in late summer, recruitment success can be inferred from the observed smaller young oyster (or spat) size (e.g., < 10-15 mm shell height) abundance patterns.

Abundance and Size:

All of the oyster beds sampled in Broad Creek and the Okatee River had a relatively low relative abundance of the larger oysters that were of harvestable size (>76 mm or 3" shell height, Figures 5.14, 5.15). Additionally, only the lower stations in each drainage system (O-4 to O-6) had large numbers of small spat less than 15 mm. A comparison of mean oyster size (shell height in mm) showed significant differences ($p < 0.0001$) among the stations (Figure 5.16), with a few clear patterns. Most of the stations (Okatee stations O-2 to O-6, Broad Creek stations O-2, O-3, O-6) were not significantly different. For example, Station O-1 in the Okatee River had the largest oysters out of all 12 stations, with station O-1 in Broad Creek next in size. Notably, both of these sites, and station O-3 in the Okatee, had larger oysters than other populations in Beaufort, Georgetown and Charleston Counties based on recent surveys (mean sizes < 30-35 mm). The mean size of oysters at station O-1 in the Okatee River was also significantly larger than the mean sizes observed at all other stations sampled in both drainage systems, with the exception of station O-1 in Broad Creek and station O-3 in the Okatee River. Station O-1 in Broad Creek had the second largest average sized oysters and they were significantly larger in size than those at stations O-3 to O-5 in Broad Creek and stations O-5 and O-6 in the Okatee. Oysters at Station O-4 in Broad Creek were the smallest on average, being significantly smaller than all other stations except for Stations O-5 in both Broad Creek and the Okatee River.

The total number of live oysters varied considerably in each system, ranging from 38-1,137 individuals/quadrat (or 0.143 m^2) in Broad Creek and 45-727 individuals in the Okatee River. When the five replicate quadrats were summed by station, the total number of oysters in Broad Creek varied from a low of 803 at the uppermost station (O-1, a restricted site) to over 3,971 individuals at station O-5, which had an "open" harvesting status. In the Okatee River, total abundances ranged from a low of 384 at the uppermost station (O-1-restricted site) to over 2,374 individuals at station O-5, a station also designated as "open" to harvesting. In general, mean oyster abundances were greater in Broad Creek than in the Okatee River but mean sizes of the oysters were larger on average in the Okatee River (Figure 5.16).

Size-Frequency Analysis:

A statistical analysis of curve intercept for the size-frequency data showed significant differences between the two drainage systems and between sites within each drainage system ($p < 0.006$ and $p < 0.001$, respectively), with Broad Creek stations having significantly higher values than stations in the Okatee. Stations O-4 to O-6 also had significantly higher intercepts and slopes ($p < 0.01$ for both) than Stations O-1 to O-3 (Appendix 5.3a). The slope of the size-frequency curves was also significantly different among stations ($p < 0.0001$), but not between river systems ($p > 0.1$). These intercept and slope findings suggest that the oyster populations in the lower three stations in each

river system had more and smaller oyster individuals than those in the upper three stations. Most of these smaller oysters were perhaps only 1-2 years old.

Statistical comparisons of the size-frequency curve slopes and intercept values within each drainage system also showed some differences (Appendix 5.3b). In Broad Creek there was a significant ($p = 0.0004$) effect of station on both the intercept and slope values. Intercept values at the lower stations O-4 to O-6 were significantly greater than at station O-1. Intercept values at stations O-2 and O-3 were not significantly different from any of the other Broad Creek stations. Slopes at stations O-1 to O-3 were significantly greater than at station O-6. Slopes at Stations O-4 and O-5 were not significantly different from any of the other stations. In the Okatee, there was also a significant station location effect on both slope and intercept values ($P < 0.001$). Stations O-4 to O-6 had significantly higher intercept values than stations O-1 to O-3. Stations O-1 and O-2 had significantly higher slope values than those observed for station O-4 to O-6. The slope at station O-2 was also higher than the slope at O-3. This analysis suggests that there are relatively clear differences between the uppermost and lowermost stations within each watershed. The comparison of population patterns among the two watersheds are less distinct. This could be due to harvesting history, recent disturbance, available substrates, and food availability as indicated by chlorophyll-a concentrations.

Oyster Diseases:

Perkinsus marinus (Dermo)

Our evaluation of the native oysters collected from the Okatee River and Broad Creek revealed *Perkinsus marinus* infections at all stations (Figure 5.17 , Appendix 5.4). This is typical of what we have observed across South Carolina in previous studies (Bobo et al., 1997; Appendix 5.5). In Broad Creek, mean infection intensity levels ranged from 0.76 at station O-5 to 2.04 at station O-3. For the Okatee River sites, mean infection levels ranged from 1.40 at station O-4 to 2.28 at station O-3. The percent of oysters infected (also called prevalence) ranged from 36% to 100% in Broad Creek and 84% to 96% in the Okatee River.

Initially, we used a two-way ANOVA with river system and station as main effects. For *P. marinus*, results were as follows: for prevalence, a significant main effect was detected for station ($p < 0.001$), but not for rivers ($p = 0.2$); for intensity levels, a significant main effect was detected also for station ($p < 0.02$), but not for rivers ($p = 0.61$). For both, however, a significant interaction was observed between river and station ($p < 0.0001$).

Further statistical comparisons indicated that there was a significant difference in the percentage of oysters infected with *P. marinus*, among the Broad Creek stations ($p < 0.001$, K-W). Station O-5 had significantly fewer infected oysters than stations O-1, O-4, O-4 (Figure 5.17 A). For mean infection intensity, significant differences (one-way ANOVA, $p < 0.001$) were detected among stations. Station O-5 had significantly lower intensity levels than all other stations, with the exception of station O-2. Finally, station O-2 was also different than station O-6 (Bonferroni Test). Overall, Broad Creek sites

were well within the observed and typical range for observed in South Carolina oysters (Appendix 5.5).

In the Okatee River, the percentage of oysters infected with *P. marinus* was not statistically different among the six sites ($p = 0.884$, K-W; Figure 5.17 B). Although there was a marginally significant difference observed overall among the sites in the mean infection intensity levels ($p < 0.05$, K-W), Dunn's pair-wise comparison tests could not distinguish a significant difference among any two sites compared. As with Broad Creek, the Okatee River sites were well within normally observed disease levels (Bobo et al., 1997, Appendix 5.5).

Observed *P. marinus* infection levels were similar to those observed in other South Carolina oyster populations based upon historical data (Bobo et al., 1997) or based on other sampling efforts conducted during the same time period in 1997 (Shellfish Research Section). *P. marinus* prevalences are typically high (>70%) during August-September, with mean infection levels rarely exceeding 3.00 on a 0-6 scale (Bobo et al. 1997). In our 1996 MRD disease monitoring study, *P. marinus* infections were present in oysters at all of the 52 sites sampled (Appendix 5.5). Sampling for that study occurred during the same period (August and September) as this study. This is a period when the disease prevalence and intensity levels are generally the highest in South Carolina oysters (Bobo et al., 1997, Crosby and Roberts. 1990).

Disease levels were not statistically different among the sites with different DHEC harvesting designations. In Broad Creek, both the highest and lowest infection intensity levels were observed at harvestable stations. In the Okatee River, the highest Dermo infection intensity level (2.28) was observed at a restricted station (O-2) and the lowest infection intensity level (1.40) was observed at an open station (O-4).

Haplosporidium nelsoni (MSX)

Examination of native oysters for the MSX disease also revealed infections at all stations (Figure 5.18, Appendix 5.4). The percentage of infected oysters ranged from 4% to 33% in Broad Creek, with the lowest levels observed at station O-3 and the highest levels observed at O-4 (Figure 5.18 A). In the Okatee River, the percentage of infected oysters ranged from 4%, observed at Stations O-1, O-2, O-4 and O-5 to 12% at station O-3 (Figure 5.18 B).

As with *P. marinus*, we used a two-way ANOVA with river system and station as main effects examining only MSX prevalence. In contrast to Dermo, we observed a significant main effect for river system ($p < 0.013$), but not for stations ($p = 0.53$). However, a marginally significant interaction was observed between river and station ($p < 0.044$).

No statistical differences in prevalence (infection) levels were noted among either the Broad Creek or Okatee River sites ($p = 0.08$, K-W). There were also no statistically significant differences noted between the two river systems, although the Broad Creek oyster beds had slightly higher levels of MSX than Okatee River oyster beds. In terms of

infection intensities, 22 of the 148 oysters (or 15%) examined from the six Broad Creek stations were infected, and only 9 of the 149 oysters (or 6%) collected from the Okatee River had MSX. Mean infection intensities in both drainage systems ranged from rare (very few parasites observed) to heavy (more than 5 parasites/400x field).

MSX was also observed at 28 of the 52 stations (54%) sampled during our 1996 summer/fall statewide disease monitoring study (Bobo et al., 1997; Appendix 5.5). These sites included stations within Beaufort County. The mean percentage of infected oysters at those sites ranged from 0 to 32%. The percentage of oysters infected in the Broad Creek and Okatee River stations were also similar to those observed in other South Carolina oyster populations sampled in previous years (Bobo et al., 1997). During our 1994 monitoring study, *H. nelsoni* infections were observed in 11 of the 21 (or 52%) sites sampled across South Carolina, with the mean number of infected oysters ranging from 0% to 42% (Bobo et al., 1997). Finally, there was no apparent relationship between the incidence of MSX in the oyster samples versus the oyster bed status using DHEC's classification in either the Broad Creek or Okatee River drainage system.

Oyster Cellular Responses

Within the past decade, there has been an increasing emphasis on the potential use of biochemical, physiological, and histological indicators as biomarkers of exposure to or effects of anthropogenic impacts (Huggett et al., 1992; Decaprio, 1997). The underlying premise of biomarker tools is that effects at higher levels of organization (populations and communities) represent the net sum of effects on individuals that resulted from alterations in cellular and molecular responses. Therefore, cellular responses should function as indicators for identifying individuals and populations for which conditions have exceeded compensatory mechanisms and are experiencing chronic stress, which if unmitigated, may progress to severe effects at higher levels of organization.

Lysosomes are regarded as valuable indicators of pollutant-induced injury (Moore, 1994). There is a substantial body of literature validating that environmental pollutants (metals and polyaromatic hydrocarbons) cause destabilization of lysosomes (Moore, 1985; Regoli, 1992; Lowe et al., 1995; Ringwood et al., 1998a and 1998b). Glutathione (GSH) is regarded as one of the most important "first-line" defense mechanisms of cells. Glutathione depletion has been observed in mammalian as well as marine organisms, and it has been hypothesized that GSH depletion is both a signal of stress (frequently in response to metals), and a predisposing factor for increased adverse effects (Meister and Anderson, 1983; Viarengo et al., 1991; Regoli and Principato, 1995; Regoli, 1998). Lipid peroxidation (LPx) reflects damage to cell membranes from free radicals. The peroxidation process is also a source of other cytotoxic products that may damage DNA and enzymes (Kehrer, 1993; Yu, 1994).

Recent studies with lysosomal destabilization and glutathione concentrations have indicated highly significant correlations with contaminants (Ringwood et al., 1998a; Ringwood et al., In press). We have developed models in which >30% destabilized

lysosomes and GSH concentrations <400 -500 nM/g indicate stress in oysters. These threshold values have been derived from field data based on juveniles and adults from a range of salinities as well as controlled laboratory experiments. The results of studies conducted with native oysters collected from the Okatee River and Broad Creek are summarized in Appendix 5.6 and Figure 5.19. The oyster collection sites do not match up directly with the sediment contaminant data, but the sites that were closest are noted in Appendix 5.6. Most of these sites had only low levels of contaminants. However, examination of the tissue contaminants data (see next section) indicated that one or more metals were present in the oyster tissue samples at elevated levels. These metal concentrations may account for the consistently high (> 30%) lysosomal destabilization rates that we observed at most of the sites. Lysosomal destabilization was significantly elevated at only one site (O-2) relative to the site with the lowest rate. In contrast, there was no evidence of severe GSH depletion, although statistically significant lower GSH levels were observed in oysters from one site in Okatee (O-6) and 2 sites in Broad. Only one site from Okatee River (O-3) had significantly elevated LPx, but LPx levels of oysters from 4 of the 6 Broad Creek sites were significantly elevated. Although we have frequently observed high LPx levels at contaminated sites, this response has not shown good correlation with contaminants. Some of our recent data suggest that elevated LPx may also be related to dissolved oxygen stress.

The elevated levels of lysosomal destabilization along with the high tissue metal concentrations suggest that oysters throughout both systems are showing signs of impaired cellular function. Although the lysosomal data may reflect exposure to contaminants, the maintenance of relatively high GSH levels indicates that oysters are not severely stressed. If the elevated LPx levels indicate DO or oxidative stress, then Broad Creek may be experiencing more DO problems than Okatee. Overall, these results suggest that both systems are showing some signs of exposure to adverse conditions, and Broad Creek oysters may be experiencing both low level contaminant and DO stress. There is no evidence that oyster populations from either system are severely stressed, but the perturbed responses suggest that they may be susceptible to further declines in habitat quality.

Tissue Contaminant Concentrations:

No pesticides, PAHs, or PCBs were detected in any samples from either system. The only metals detected in either system were; arsenic, cadmium, aluminum, manganese, copper, and zinc (Appendix 5.7).

Arsenic was detected only at the two lower zone sites in Broad Creek; O-5 and O-6, at concentrations of 2.4 and 2.2 mg/kg wet weight, respectively (Appendix 5.7). The U.S. Food and Drug Administration (USFDA) has published guidance for deriving a level of concern for human consumption of shellfish due to arsenic and provided an example based on national figures (USFDA, 1993a). At both sites, the wet weight tissue concentration was well below the example level of concern of 86 mg/kg for chronic consumers of shellfish (15 g/person/day). These values were also well within the range

of 3.0 – 4.0 mg/kg (USFDA, 1993a) measured by the National Marine Fisheries Service (NMFS), although they were near the top of the range of 0 – 2.8 mg/kg measured by USFDA (1993a).

When converted to dry weight the resulting arsenic concentration at site O-5 was 24 mg/kg and 22 mg/kg at site O-6 (Appendix 5.7). Both of these values were greater than the calculated mean of 9.29 mg/kg computed from O'Connor (1996). The levels are also similar to those observed by Scott (NOS, unpublished) in other parts of the state. Arsenic levels are naturally elevated in South Carolina relative to other parts of the United States due to local geochemistry. Although these values are greater than the national average, the concentrations observed in the oyster tissue do not necessarily mean there is ecological degradation.

Cadmium was detected at all sites in both systems except the two upper zone sites in Broad Creek (O-1 and O-2) and ranged from 0.2 to 0.5 mg/kg wet weight (Table 5.6). USFDA has published guidance for deriving a level of concern for human consumption of shellfish due to cadmium and provided an example based on national figures (USFDA, 1993b). At all sites the wet weight tissue concentrations were well below the example level of concern of 3.7 mg/kg for chronic consumers of shellfish (15 g/person/day). The values also were well below the range of 0.9 – 1.0 mg/kg measured by NMFS (USFDA, 1993b), and within the range of 0.25 – 1.12 mg/kg measured by USFDA (1993b). Additionally, the concentrations we measured were comparable to coast-wide data collected by SCDHEC since 1980 (n = 115, median = 0.4 mg/kg, range 0.2 – 1.6 mg/kg, 90^{th} percentile = 0.7 mg/kg).

When converted to dry weight the resulting range in cadmium concentrations was 2.0 – 5.0 mg/kg, (Table 5.6, Appendix 5.7). Okatee River sites O-1 through O-4 and O-6, and Broad Creek site O-6 in Broad Creek were greater than the calculated mean of 2.68 mg/kg computed from O'Connor (1996). The values were also generally higher than we have noted in other parts of the state (Ringwood, SCDNR unpublished data; Scott, NOS unpublished data). Okatee River cadmium concentrations were significantly greater than Broad Creek ($p = 0.036$). Biological effects related to the presence of high cadmium levels in oyster tissue are not well documented, but the values we observed may be below levels that cause cellular dysfunction based on data obtained by Ringwood, who observed Cd tissue levels > 1.5 mg/kg in oysters that had high (> 30%) lysosomal destabilization.

Aluminum, manganese, copper, and zinc were detected at all sites in both systems. There is no USFDA guidance for human consumption of shellfish related to any of these metals. O'Connor (1996) indicates that aluminum and manganese are not contaminants, *per se*, because their relatively high natural concentrations in the environment are not significantly altered by human activities.

Wet weight copper concentrations ranged from 6.5 – 27 mg/kg (Table 5.6, Appendix 5.7). These concentrations were comparable to coast-wide data collected by SCDHEC since 1980 (n = 115, median = 13 mg/kg, range 3.8 – 130 mg/kg, 90^{th} percentile = 30 mg/kg. When converted to dry weight the resulting range in copper

concentrations was 64.9 – 269.6 mg/kg (Table 5.6, Appendix 5.7). Broad Creek sites O-1 through O-5, and site O-6 in the Okatee River were greater than the calculated mean of 123.75 mg/kg computed from O'Connor (1996). It was also in the high range of copper concentrations observed by Wendt et al. (1996) in creeks with high dock densities. There was no significant difference in copper concentrations between the two systems ($p = 0.098$). The generally higher copper concentrations at most sites in Broad Creek may reflect the much higher boating and marina activity in this system, since most anitfouling paints utilize copper as the bioinhibitor. Biological effects related to the presence of high copper levels in oyster tissue are not well documented, but concentrations greater than 95 mg/kg were found in oysters having cellular dysfunction with respect to having greater than 30% lysosomal destabilization (Ringwood, unpublished). Fresco (1997) found similar elevated Cu uptake in oysters from highly urbanized Murrells Inlet, with highest uptake observed at sites with marinas and high dock density. The high Cu levels observed at station O-6 in the Okatee may have resulted from Cu enrichment due to metal based fungicides used in vegetable farming. Elevated Cu levels have been found in sediments and oysters at sites downstream of major tomato farming areas (Scott et al., 1999)

Wet weight zinc concentrations ranged from 140 – 660 mg/kg (Table 5.6, Appendix 5.7). The value of 660 mg/kg was measured at the Okatee River site O-1 and is in the top 10% of the samples measured coastwide by SCDHEC since 1980 (n = 115, median = 280 mg/kg, range 14 - 1200 mg/kg, 90^{th} percentile = 580 mg/kg). When converted to dry weight the resulting range in zinc concentrations was 1398 - 6591 mg/kg (Table 5.6, Appendix 5.7). All sites except Broad Creek site O-6 were greater than the calculated mean of 1950 mg/kg computed from O'Connor (1996). There was no significant difference in zinc concentrations between the two systems ($p = 0.076$). Biological effects related to the presence of high zinc levels in oyster tissue are not well documented, but concentrations greater than 1330 mg/kg were found in oysters having cellular dysfunction with respect to having greater than 30% lysosomal destabilization (Ringwood, unpublished).

Evaluation of Changes in Bed Acreage Over Time:

Results of the survey of oyster beds sampled in this study to estimate population size, total shellfish volume, and live oysters are presented in Table 5.7 and compared with similar data obtained in 1984-1985. The change in bottom area or "footprint" of the intertidal bed did not always correspond to the change in shellfish volume, either with respect to total volume (includes dead shell and substrate) or with respect to "live volume". In Broad Creek, the six station total indicated approximately a 21% *increase* in area, but a 17% *decrease* in live volume. In comparison, the cumulative estimate obtained from the six stations in the Okatee River showed a 14% *decrease* in area, and a 41% *decrease* in live volume. In some locations (e.g. O-6 in Broad Creek) the oyster population footprint decreased in area, but increased in total live volume. This trend results from changes in densities, which are characterizations of standing crop spatial dispersions. Oyster densities and the percentage of bed that consists of live oysters is

sometimes dependent on whether the beds are open to harvesting or not. In Broad Creek, the upper four beds showed either no change in size (O-2) or an increase in size. Three of these beds were closed to shellfish harvesting at the time of the study. The two lowest beds (O-5, O-6) were open for harvest as a culture permit. Similarly, the four stations in the lower portion of the Okatee River (O-3 to O-6) were also open to shellfish harvests as a culture permit. All but one showed a decrease in size and all four showed a decrease in total live shell volume, perhaps due to harvesting pressure.

Mussel Abundance and Biomass:

Mussels (primarily *Geukensia demissa* and *Brachidontes exustus*) varied significantly among the 12 stations, with total abundance (sum of 5 samples/station) ranging from 0 at Okatee site O-6 to 707 individuals at the Broad site, O-6 (Figure 5.20; Appendix 5.8). Total wet mussel biomass (sum of 5 samples/station) ranged from 0 at Okatee site O-6 to 214 g at the Broad Creek site O-6. Mussels were rare at all of the Okatee stations, with total abundances ranging from only 0 to 14 individuals. In Broad Creek, the total abundance of mussels ranged from 8-707 individuals. Based on water quality data, the sites sampled in this study did not differ significantly in physical factors (salinity, temperatures) nor in other indicators of habitat quality. Hence, the large difference we observed between river systems is unclear. Previously, we have noted that mussels were often rare at sites with poor quality growth (e.g. Warsaw Flats-Beaufort Co.) and poor water quality (e.g., Toler's Cove-Charleston Co.).

General Conclusions of Oyster Population Assessment:

An integrated summary of oyster bed conditions that we observed in Broad Creek and the Okatee River is provided in Figure 5.21. In general, the beds that showed the greatest evidence of some degradation in condition were located in the headwater (upper zone) portions of both drainage basins, when the four primary measures of oyster condition were considered (size and density, disease, cellular response, tissue contaminant levels). None of the beds in either the Okatee River or Broad Creek coded as good (green) for all measures, but it should be noted that this may or may not be due to environmental stress at many of the sites.

The overall size and density of oysters was generally good with respect to current SC conditions in both drainage systems and greater in Broad Creek compared to the Okatee River, which is most likely due to the more restrictive harvesting in Broad Creek. Density and evidence of good recruitment to the beds (indicating bed sustainability) was lowest in the headwaters, where salinity variance is greatest. Oysters cannot survive well at salinities less than 10 ppt (Shumway, 1996). We used the overall number of oysters, which incorporated recent oyster recruitment, as the measure of bed density with sites having > 1,000 live oysters in the five quadrat samples coding as good. Oyster populations were rated 'good' for all Broad Creek sites, with the exception of the uppermost station, 0-1. Similarly, all three lower stations in the Okatee River (O-4, O-5, 0-6), rated as 'good', but the uppermost three were all deemed 'marginal'.

Disease incidence in the oysters was generally similar in both systems and consistent with disease prevalence and infection intensity levels observed elsewhere in the state. All six Okatee sites were given a 'good' rating, whereas only 4 of the 6 stations in Broad (O-1, O-2 , O-3 and O-6) received a 'good' rating due to the higher incidence of MSX at the other sites.

The cellular responses observed in both systems were also similar, but higher than anticipated with respect to one or two of the assays considered, particularly the lysosomal destabilization assay. This may reflect responses to the tissue contaminant levels observed in oyster samples collected from both drainage systems, or to other environmental stresses such as low dissolved oxygen.

The only contaminants detected in the oyster tissue samples were four metals (Cd, Cu, Zn, and As). None of these metals were at alarming levels, but there is evidence that some of the metal concentrations were high enough to elicit sublethal responses with respect to cellular function, and cellular dysfunction was observed at all of the sites sampled in this study. The consistently higher levels of copper in Broad Creek may be reflecting the much higher boating and marina activity in that system compared to the Okatee. Most antifouling paints in use today utilize copper to inhibit the settlement of fouling organisms. The higher levels of cadmium and zinc observed in oysters collected from the Okatee River may reflect the effects of long-term agricultural runoff, since these metals are often utilized in pesticides.

Grass Shrimp Population Assessment:

The grass shrimp, *Palaemonetes pugio,* is a common inhabitant of southeastern and Gulf coast estuaries of North America. These shrimp are a major force in accelerating the breakdown of detritus in the estuary (Welsh, 1975) and are important dietary components for many fish species (Wood, 1967). In South Carolina estuaries, *P. pugio* occur year round at densities ranging from < 1000/50 m of stream in winter to 28,000/50 m of stream in summer. Grass shrimp may comprise 56% of the total macrofaunal stream density on an annual basis (Scott et al., 1992).

Grass shrimp are external brooders with the developing eggs attached to the pleopods of the female until hatching. This makes ecotoxicological population measurements of the adults, larvae and embryos of this species suitable for use in field contaminant assessments in several ways. First, adults can be assessed directly and their sensitivity to waterborne and/or sediment-associated contaminants ascertained. Secondly, during the two-week brooding period, embryos carried by the female are directly exposed to any contaminants present in the water column so that brood counts of eggs in gravid females can be utilized to predict effects in embryos. Finally, enumeration of larval abundance makes determination of the entire life cycle complete. Comparative toxicity testing of different life history stages has indicated that for many environmental

contaminants, embryo, larval, post-larval and adult stages have similar sensitivities (; Baughman et al., 1989; Scott et al., 1992; Key, 1995; Lund, 1997).

Scott and co-workers at the NOAA/NOS Charleston Lab have utilized grass shrimp population abundance as an effective method for assessing impacts of agricultural non-point source (NPS) runoff of endosulfan, azinphosmethyl and fenvalerate (Patterson, 1985; Hampton, 1986; and Scott et al., 1992). This species has also been an effective indicator of urban NPS runoff effects (Fulton et al., 1993; Fulton et al., 1996; Finley et al., 1998). Finley et al. (1998) demonstrated that urban and agricultural NPS runoff had a significant effect on adult density and reproduction (e.g. delayed brood and altered sex ratios). In urban areas these effects were correlated with exposure to PAHs and alterations in physicochemical water quality (e.g. salinity and dissolved oxygen) associated with urban development. In agricultural areas, effects were correlated with pesticide runoff of azinphosmethyl. Porter et al. (1995) found that GIS and spatial statistical methods (e.g. krieging) were useful in generating estuarine-wide grass shrimp abundance maps for both adult and larval grass shrimp in urbanized Murrells Inlet, which were reduced by > 85% estuarine wide when compared to pristine North Inlet, a NOAA NERRS site. Additionally, it was possible through data layer overlays of land-use and sediment chemical contaminant data to correlate reduced grass shrimp abundances with known pollution sources (e.g. highway runoff and marinas) within the estuary.

Specific objectives of the grass shrimp population assessment were as follows:

1. Enumerate adult grass shrimp abundance, biomass and sex ratios within tidal creek and river habitats along the length of each drainage basin;

2. Quantify the reproductive output of adult female grass shrimp within tidal creek and river habitats along the length of each drainage basin;

3. Enumerate the larval grass shrimp abundance within tidal creek and habitats along the length of each drainage basin;

4. Compare adult and larval grass shrimp population metrics (e.g. biomass, abundance, sex ratio, egg production/female) between each sub-habitat within each drainage basin; and

5. Compare adult and larval grass shrimp population metrics (e.g. biomass, abundance, sex ratios, egg production/female) between each drainage basin.

Methods:

Grass shrimp (*Palaemonetes* spp.) populations were sampled using a push net at a total of 30 sites, with 15 sites located within each drainage basin (Figures 5.1, 5.2). The sampling procedure, which is described by Scott et al. (1992), involves a push net

sampling method that is a modification of the approach described by Welsh (1975). Within each basin, the push netting was conducted at sites that approximated a gradient of pollution and landscape ecology (e.g. tidal creek --> river -->intertidal sites). Previous research on grass shrimp population dynamics (monthly sampling for 12 months) have been conducted around sites with known pollution sources (e.g. Koppers, Diesel and Shipyard Creeks) in Charleston Harbor. Additionally, estuarine wide sampling (e.g. one time random sampling) has been conducted at 30 sites in suburbanized Murrells Inlet, 42 sites in Charleston Harbor, 30 sites in North Inlet, and 34 sites in the ACE Basin. Thus, an extensive historical database (1985-present) exists for grass shrimp population dynamics within South Carolina that was used to compare results in this study.

At each site, three consecutive 25-m lengths of creek were marked with PVC stakes and sampled 2.5 hours prior to low tide. Sampling was conducted either on foot or from a 3-m plastic catamaran with an electric trolling motor. Tows were made along each bank using a push net with a mouth opening of 1,009 cm^2 (approximately 25-cm length x 40-cm width). A 360-μm plankton net 20-cm deep was attached to the back of each net. Tows were made against the tidal current along each bank at, or near the mid-tide period. The contents of the tows from each bank were combined to produce three replicate samples per site. Adult (> 15 mm) and sub-adult (<15 mm) grass shrimp collected in the net were preserved in > 50% ethyl alcohol and stored until processed. Comparisons of tows made on foot versus those made while trolling have found no statistical differences between the two collection methods (Scott, unpublished).

The following parameters were measured for adult grass shrimp: (1) total abundance (*P. pugio*, *P. vulgaris*, *Penaeus* sp. and other species as #/m of stream); (2) total biomass (*P. pugio*, *P. vulgaris*, Penaeus sp. and other species as g/m of creek); (3) *P. pugio* abundance (#/m of stream); (4) *Palaemonetes pugio* biomass (g/m of stream); (5) abundance of male, non-gravid female and gravid female *P. pugio* (#/m of stream); (6) sex ratio of *P. pugio* (% males: nongravid females: gravid females); (7) the number of eggs/female (#/ female) in *P. pugio* and (8) larval *P. pugio* abundance (#/m of stream)

Statistical comparisons of each metric were made, using both intra-site (tidal creek versus river versus intertidal within the Okatee River and Broad Creek and inter-site (tidal creek, river, intertidal) and pooled comparisons for the Okatee River versus Broad Creek) using both non parametric (Wilcoxon; Mann Whitney; Kruskal Wallis) and parametric (ANOVA, Dunns , and Dunnetts) procedures. An alpha level of 0.05 was used to determine statistical significance among sites. Multiple regression analysis was used to assess the effects of variables such as overall sediment quality (sediment toxicity tests, ERM Quotients and ammonia concentrations), overall physicochemical water quality, specific water quality parameters (DO and salinity), specific sediment quality guidelines (ERM Quotients), and benthic faunal densities on different grass shrimp metrics. Both parametric (Linear regression with appropriate data transformation) and non-parametric (Spearman and Pearson Correlations) methods were used. Both correlation coefficients (R^2 and Rho/Tau) were calculated along with P values to determine if regressions were significant ($p < 0.05$).

Statistical comparisons of the grass shrimp population metrics with results from laboratory analytical chemistry results were made to indicate whether population metrics were correlated with chemical contaminant concentrations in sediments, physicochemical water quality alterations or loss of physical marsh/tidal creek habitat. Site and drainage basin comparisons were also performed using various parametric and non-parametric methods to determine patterns for each of the major population parameters. Analysis of grass shrimp indicated the following composition: *P. pugio* 75%, *P. vulgaris* 25%, and *P. intermedius* < 1%. Since *P. pugio* was the dominant species observed in both watersheds all grass shrimp results were classified as *P. pugio* for statistical analysis.

Findings:

Adult Grass Shrimp:

Comparisons of adult grass shrimp abundance indicated that generally there were no significant differences within tidal creek, river and intertidal habitats between Broad Creek and the Okatee River (Figure 5.22, Table 5.8, Appendix 5.9). Pooled stations comparisons (tide creek + river + intertidal) within each watershed indicated a significant ($p < 0.032$) difference in abundance (Figure 5.22), with higher abundances observed in Broad Creek than in the Okatee River. A closer inspection of these data, however, generally indicated that mean abundances and associated variances were quite comparable. The statistical difference noted in pooled abundance may have resulted from the non-parametric procedures used for statistical analysis, which compare median versus mean values. In individual multiple station comparisons, only Broad Creek T-5 had significantly ($p < 0.027$) higher abundance than Okatee River T-4 (Figure 5.23).

P. pugio biomass showed a similar trend, as between watershed comparisons indicated that generally there were no significant differences in tidal creek, river and intertidal habitat comparisons between Broad Creek and the Okatee River (Figure 5.22, Table 5.8). Pooled stations comparisons (tidal + river + intertidal) within each watershed indicated significantly ($p <0.029$) higher biomass in Broad Creek when compared to the Okatee River (Figure 5.22). A closer inspection of these data however, generally indicated that mean biomass and associated variances were quite comparable. As noted for total abundance comparisons, the statistical difference noted in pooled biomass may have resulted from the non-parametric procedures used for statistical analysis, which compares median versus mean values. In individual station comparisons (Figure 5.23), tidal creeks stations T-5 and T-3 in Broad Creek had significantly ($p< 0.0001$) higher biomass than other stations in Broad Creek (T-1,T-2, T-3, T-4 and T-6) and the Okatee River (T-1, T-2,T-3, T-4, T-5 and T-6). At river sites, stations R-2 and R-1 in Broad Creek and stations R-2 and R-3 in the Okatee River had significantly higher biomass than other stations in Broad Creek (R-3, R-4, R-5 and R-6) and the Okatee River (R-1, R-5 and R-6). At intertidal sites, stations I-1 and I-6 in Broad Creek had significantly higher biomass than stations I-1 and I-6 in the Okatee. Similarly, the intertidal station I-4 in the Okatee had significantly higher biomass than the Broad Creek station I-4 and the Okatee stations I-2 and I-6).

The average *P. pugio* size (biomass/density = g/individual) was not significantly different in inter-watershed comparisons of tidal creek, river and intertidal habitats between Broad Creek and the Okatee River (Figure 5.22, Table 5.8). Pooled stations comparisons (tidal + river + intertidal) within and between each watershed indicated that average *P. pugio* size was not significantly different in Broad Creek when compared to the Okatee River (Figure 5.22). In individual station comparisons (Figure 5.23), tidal creek stations were significantly ($p < 0.001$) different in Kruskal-Wallace comparisons between Broad Creek and the Okatee River, although no individual stations were significantly different in multiple pair-wise comparisons.

The percent gravid female *P. pugio* was not significantly different in inter-watershed comparisons of tidal creek and intertidal habitats between Broad Creek and the Okatee River (Figure 5.24, Table 5.8). Mann Whitney U comparisons of river stations were significantly ($p < 0.002$) different, with a higher proportion of gravid females in Broad Creek than the Okatee River. Pooled stations comparisons (tidal + river + intertidal) within and between each watershed indicated that the % gravid female *P. pugio* was not significantly different in the two drainage systems (Figure 5.24). In individual station comparisons (Figure 5.25), the % gravid females was found significantly ($p < 0.0197$) different at river sites using ANOVA and Kruskal-Wallace procedures, although no individual stations were significantly different in pair-wise comparisons (Dunn's).

The number of eggs per female *P. pugio* was not significantly different in inter-watershed comparisons of tidal creek, river and intertidal habitats between Broad Creek and the Okatee River (Figure 5.24, Table 5.8). Pooled stations comparisons (tidal + river + intertidal) within and between each watershed indicated that the number of eggs per female *P. pugio* was not significantly different in Broad Creek when compared to the Okatee (Figure 5.24). When comparisons among stations were made (Figure 5.25), the number of eggs per female was not significantly different among tidal creek sites, but in river and intertidal site comparisons, occasional differences were observed.

Larval Grass Shrimp:

The number of larval *P. pugio* was not significantly different in inter-watershed comparisons of tidal creek, river and intertidal habitats between Broad Creek and the Okatee River (Figure 5.24, Table 5.8, Appendix 5.9). Pooled stations comparisons (tidal + river + intertidal) within and between each watershed indicated that the number of larval *P. pugio* was not significantly different between the two drainage systems (Figure 5.24). In individual station comparisons (Figure 5.25), the number of larval *P. pugio* was not significantly different in tidal creek comparisons between Broad Creek and the Okatee; however, river and intertidal site comparisons were significantly different between Broad and Okatee sites using ANOVA and Kruskal –Wallace tests ($p < 0.004$ and 0.026, respectively, Figure 5.25). No individual site differences were noted in the tidal creeks, but at river and intertidal sites occasional differences were observed between Broad Creek and the Okatee River.

Chemical Contaminant Effects on P. Pugio:

Additional statistical analyses of adult and larval grass shrimp metrics were conducted in which stations in both drainage systems were classified as undegraded (cumulative ERM Quotient < 0.024), marginally degraded (cumulative ERM Quotient > 0.024 ≤ 0.068) and degraded (cumulative ERM Quotient > 0.068) based on criteria described by Hyland et al. (1999). The results indicated that there were no differences in adult grass shrimp abundance, biomass, average size, sex ratios and egg production/female in comparisons of degraded and potentially degraded versus undegraded sites (Figure 5.26). Only larval grass shrimp density was significantly ($p < 0.0001$) different, with degraded sites having lower densities than undegraded sites.

Overall Assessment of Grass Shrimp Populations:

Figure 5.27 depicts an integrated assessment of selected adult and larval grass shrimp metrics at each site within Broad Creek. Significant reductions in biomass were observed in Broad Creek at 5 out of 15 sites and no site had more than one of the four selected grass shrimp metrics reduced when compared to the Okatee River. Six out of 15 sites in Broad Creek had significantly higher biomass than the Okatee sites. One out of 15 sites in Broad Creek had significantly higher biomass and abundance when compared to the Okatee sites. There were no significant differences in the % gravid female sex ratios and larval grass shrimp abundance noted in comparisons of the two drainage systems.

Figure 5.28 depicts an integrated assessment of selected adult and larval grass shrimp metrics at each site within the Okatee River. Significant reductions were noted in biomass when compared to Broad Creek at 11 out of 15 sites (73.3%), but only one site (T4=6.7%) had significant reductions in more than one of the four selected grass shrimp metrics when compared to Broad Creek. Three out of 15 sites (20%) in the Okatee had significantly higher biomass than Broad Creek sites. There were no significant differences in the % gravid female sex ratios and larval grass shrimp abundance noted in comparisons between systems.

Thus, in general grass shrimp populations within both drainage systems seem quite comparable, despite the presence of chemical contaminants, alterations in hydrography and water quality and habitat modifications within each system. When grass shrimp abundance in the two drainage systems were compared with grass shrimp abundance in North Inlet, a NOAA National Estuarine Research Reserve and Sanctuary (NERRS) site, and another pristine site located in Leadenwah Creek in the North Edisto River, numerical abundances were slightly higher in both Broad Creek and the Okatee River (Figure 5.29). However, these differences were not statistically different.

Finfish Collections:

The mummichog (*Fundulus heteroclitus*) is a common and ecologically important fish inhabiting estuaries from the Gulf of St. Lawrence to Texas (Scott and Scott, 1988). As both consumer and prey, the mummichog provides an important link in energy transfer from the marsh surface to subtidal systems (Radtke and Dean, 1979; Weisberg et al., 1981). Its tolerance for variations, even extremes, in temperature, dissolved oxygen, and salinity allow it to thrive throughout estuaries within its geographic range (Bigelow and Schroeder, 1953; Abraham, 1985; Scott and Scott, 1988). In contrast with the wide distribution of the species, individual fish have relatively small home ranges, often on the order of tens of meters (Lotrich, 1975). Consequently, mummichogs have been evaluated as indicators of conditions in the small area that they inhabit (Vogelbein et al., 1990, Holland et al., unpublished data). The objectives of this phase of the study were to compare mummichogs collected from the Okatee River and Broad Creek with respect to the following:

1. A condition index which incorporates size and weight,
2. Prevalence of gross abnormalities, and,
3. Sex ratios of mummichog populations.

Methods:

Mummichogs were collected from the same section of each creek where sediments were collected for analysis of benthic invertebrates (Figures 5.1, 5.2). Commercially available minnow traps constructed of galvanized steel with conical openings approximately 2 cm in diameter were used to collect the mummichogs. Each trap was baited with a 170-gram can of tuna (in water) with holes poked in the top. Typically, traps were fished while the sediment samples were collected, but if sufficient number of mummichogs were not caught they were fished overnight. Mummichogs were stored on ice immediately following collection and frozen upon return to the laboratory.

Mummichogs were processed in the laboratory. Total and standard lengths were measured to the nearest millimeter. Weights were recorded to the nearest 0.1gram. A condition index ([weight /standard length^3]*100,000) was calculated for each mummichog. Sex was determined by coloration, and when necessary, examination of the gonads. All fish were inspected for gross external morphological abnormalities.
.

Findings:

A total of 1367 mummichogs were collected from the 11 tidal creeks sampled (Appendix 5.10). Mummichogs were not collected from T-6 in Broad Creek, which is located near Calibogue Sound in the lower section of Broad Creek. As a result, this creek

was excluded from the analysis. At least 44 mummichogs were collected from each creek.

While abnormalities are present even in healthy populations of fish, rates are generally low. Among the mummichogs we collected, missing eyes and damaged caudal fins were the most commonly observed abnormalities (Table 5.9). Although both conditions represent serious problems for the individual fish, the overall rate of these conditions in our samples was low (about 1.5%). This rate is similar to that observed by Holland et al. (unpublished data) for tidal creeks in the Charleston, SC area. Also, rates of abnormalities among creeks within the Broad Creek and Okatee River were not significantly different (t-test, $p = 0.556$). Consequently, these conditions probably did not significantly impact populations of mummichogs within the creeks that we sampled.

Sex of most mummichogs (96.4%) was determined by cursory inspection. Mummichogs with no obvious external indication of sex were dissected. For a small proportion of the animals, sex could not be determined following dissection. These mummichogs, which accounted for only 1.3% of the total animals, were considered immature. Generally, populations of mummichogs we sampled had slightly more females than males (51-62%, Table 5.9). However, in the Okatee River T-1 and T-3 these percentages exceeded 75%. Overall, sex ratios of mummichogs were not significantly different between rivers (t-test, $p = 0.570$).

In general, the condition of mummichogs was similar to that observed by Holland (unpublished data). The condition index averaged 2.38 in the current study (Table 5.9) and 2.40 for Holland. Holland found that the condition index of mummichogs from degraded creeks was significantly lower than mummichogs from creeks that were not degraded. In the present study, we found a difference in condition of mummichogs between creeks of the Okatee and Broad ($p = 0.015$) watersheds. Larger mummichogs were collected in the tidal creeks of the Broad Creek where the three creeks producing the largest mummichogs were found. These included creeks from the upper (T-1, T-2) and lower section (T-5) of Broad Creek. Four of the five creeks producing mummichogs with the lowest average condition index were located in the Okatee River. These included all creeks from the upper and middle sections of the Okatee River (T-1, T-2, T-3, and T-4). In Broad Creek, the site with the lowest index was T-4. These creeks in both the Okatee River (T-1 to T-4) and Broad Creek (T-6) also had the lowest grass shrimp abundance and biomass. Grass shrimp are known prey items for mummichogs (Scott et. al., 1992).

Overall Conclusions Regarding Mummichog Populations:

Our initial assumption that impacted creeks would have mummichogs with lower condition indices contradicts our results. In fact, the tidal creeks of Broad Creek, which had greater overall evidence of anthropogenic stress than those of the Okatee, produced mummichogs with a higher condition index. This result may be accounted for through closer inspection of contaminant and water quality data. While the degraded creeks in

the Holland study were heavily polluted with PAHs and metals, levels in the creeks of our study were much lower, often below threshold bioeffects concentrations. The degraded creeks of the Broad and Okatee systems, in contrast, were organically enriched. This type of enrichment, which can lead to oxygen depletion, results from excessive production due to an overabundance of nutrients. As a species tolerant of low dissolved oxygen concentrations, the increased production may actually benefit the mummichog. Interestingly, a comparison of mummichog condition index with minimum dissolved oxygen value shows a high correlation ($r^2 = 0.635$, $p = 0.036$).

These results suggest that mummichog condition index alone does not provide a clear measure of creek health. However, a more substantial database would be required to confirm or refute this hypothesis. Since interpretation of mummichog condition index may only be possible when data on other important variables (e.g. contaminant concentrations, water quality, condition of food resources, etc.) are available, the examination of these other supporting data sets should be sufficient to evaluate the condition of a creek.

General Conclusions Regarding the Overall Condition of Biota

A basic premise of this study was that the Broad Creek watershed and associated estuarine habitat represented a suburbanized, disturbed ecosystem, and the Okatee River watershed and associated estuarine habitat represented an undeveloped, forested reference ecosystem. The physical-chemical and biological data collected for tidal creeks and open-water habitats in these systems, however, do not support this premise at most of the sites sampled. Rather, our overall assessment of the key invertebrate communities and indicator species sampled in this study indicate that both Broad Creek and the Okatee River generally have healthy biological assemblages that are consistent with other non-degraded estuarine sites that have been sampled in South Carolina.

We did detect some evidence of biological stress that was localized and primarily associated with the headwater portions of both systems. These biological data, combined with the environmental quality data collected at all of the sites, indicate that the headwater areas have degraded environmental quality that may limit the capacity of these systems to support designated uses and perform critical ecological functions. The evidence of degraded environmental conditions was greater in the headwaters of Broad Creek than in the headwaters of Okatee River. In the headwaters of Broad Creek, we found both water and sediment quality problems including exposure to “below normal” low dissolved oxygen levels, exposure to chemically contaminated sediments, and “above normal” salinity variation. Contaminant problems and associated evidence of some biological stress were also found at most of the intertidal mud flat and oyster reef sites sampled throughout Broad Creek; however, these effects generally did not result in a severe degradation of the resources sampled. Rather, the response was limited to evidence of sublethal (cellular) stress in the oyster assemblages, and broader population and community changes in the benthos in the form of communities that were dominated by species known to be tolerant of pollution and other environmental stresses. In many

cases, the biological responses we measured in Broad Creek were better than those sampled in the Okatee River (e.g. several grass shrimp metrics, oysters density and size in the lower reaches of Broad Creek, and mummichog size and condition).

In the headwaters of the Okatee, the poor environmental quality was mainly attributable to "above normal" variation in salinity and the biological response was primarily limited to evidence of stress in the benthic invertebrate assemblages inhabiting tidal creek habitats in headwater portions of the River. Other evidence of biological stress was limited to higher than anticipated levels of a few metal contaminants in oyster tissue throughout the drainage system, along with evidence of a sublethal (cellular) response in the oyster populations sampled at those sites. The tissue contaminant levels observed may reflect the effects of non-point source runoff from agricultural fields. Additional runoff from urban developments planned or being built in this watershed may lead to further degradation of these resources, unless both urban and agricultural inputs are controlled.

Of the four types of biological resources sampled, the benthic macrofauna and oyster condition (primarily cellular measures) appeared to show the greatest sensitivity as early warning measures related to anthropogenic stress. These resources should be included in any future assessments of Broad Creek and the Okatee River. Other measures of shellfish condition not included in this study (such as growth rates and other measures of individual condition) should also be considered. While the grass shrimp appeared to be less sensitive to the environmental conditions observed in these systems, they have proven to be sensitive indicators of environmental condition in other areas where they have been studied. The lack of any obvious differences observed between the two drainage systems in the grass shrimp metrics most likely indicates that these systems are fairly similar with respect to their overall quality to support these and other crustacean assemblages. Until more evidence is available to support the usefulness of evaluating condition in mummichog populations, we would not recommend including this measure in future biological assessments.

The lack of sensitivity in grass shrimp and mummichog populations may, in part, be related to their epibenthic nature, residing in the water column at or above the sediment surface. In contrast, the benthic organisms we sampled primarily live within the sediment. Similarly, oysters are in more direct contact with sediments scoured off the bottom and feed on suspended particulate matter, which includes filtering sediment particles. As a result of these ecological differences, benthic and sedentary epibenthic organisms (e.g. oysters) would have greater exposure to sediment-bound contaminants than epibenthic motile organisms, such as grass shrimp and mummichogs. Our results suggest that contaminant effects are confined to sediment exposure and concentrations were not sufficiently high to cause significant exposure to the grass shrimp and mummichogs. However, the benthic community and oyster effects observed provide an "early warning" indicator of ecological effects that should not be ignored. These effects were confirmed at many of the sites by the sub-lethal toxicity assays (e.g. seed clam and Microtox), which provides another early warning signal.

Broad Creek

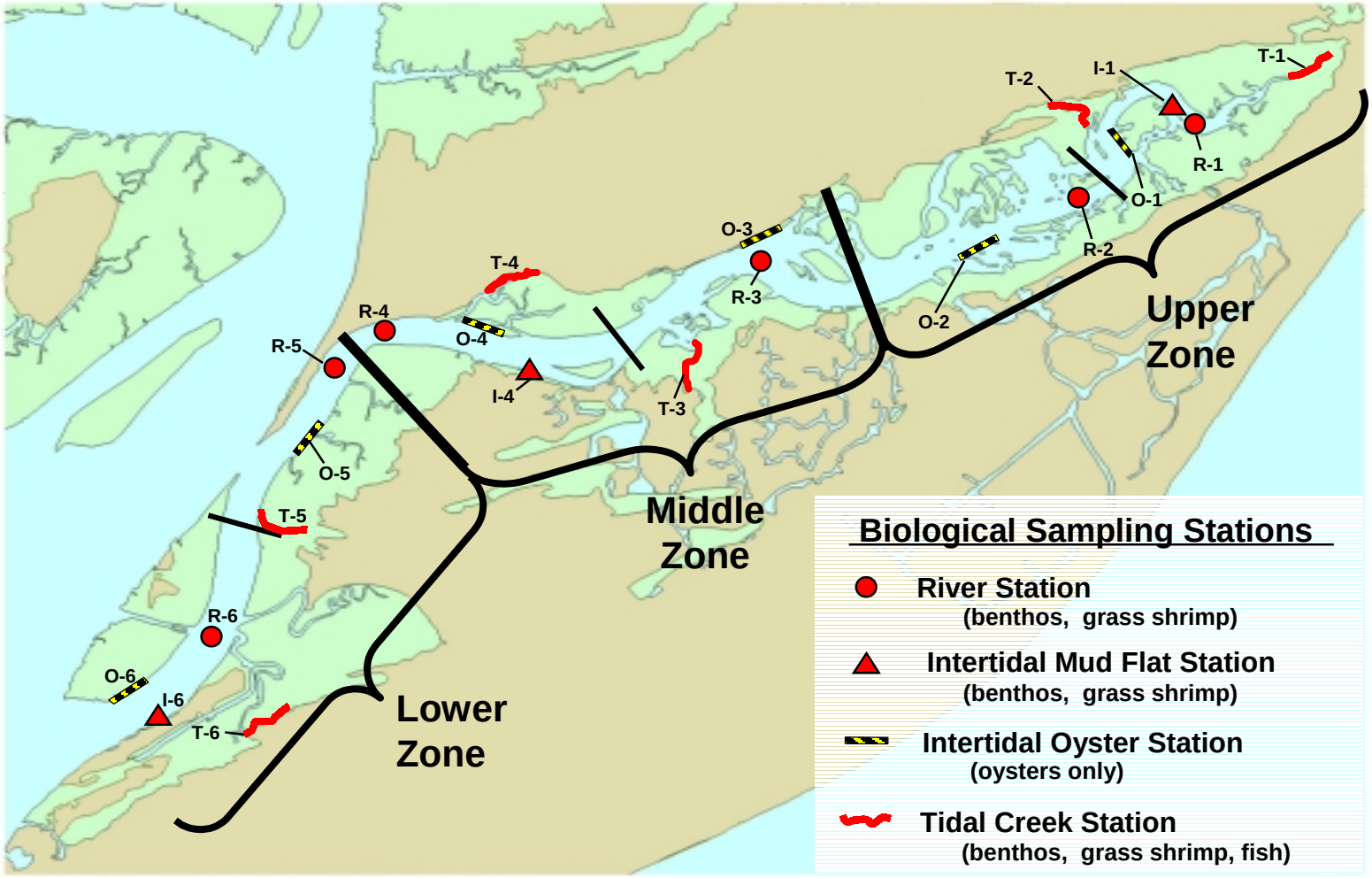


Figure 5.1. Map of Broad Creek stations sampled for biota in 1998.

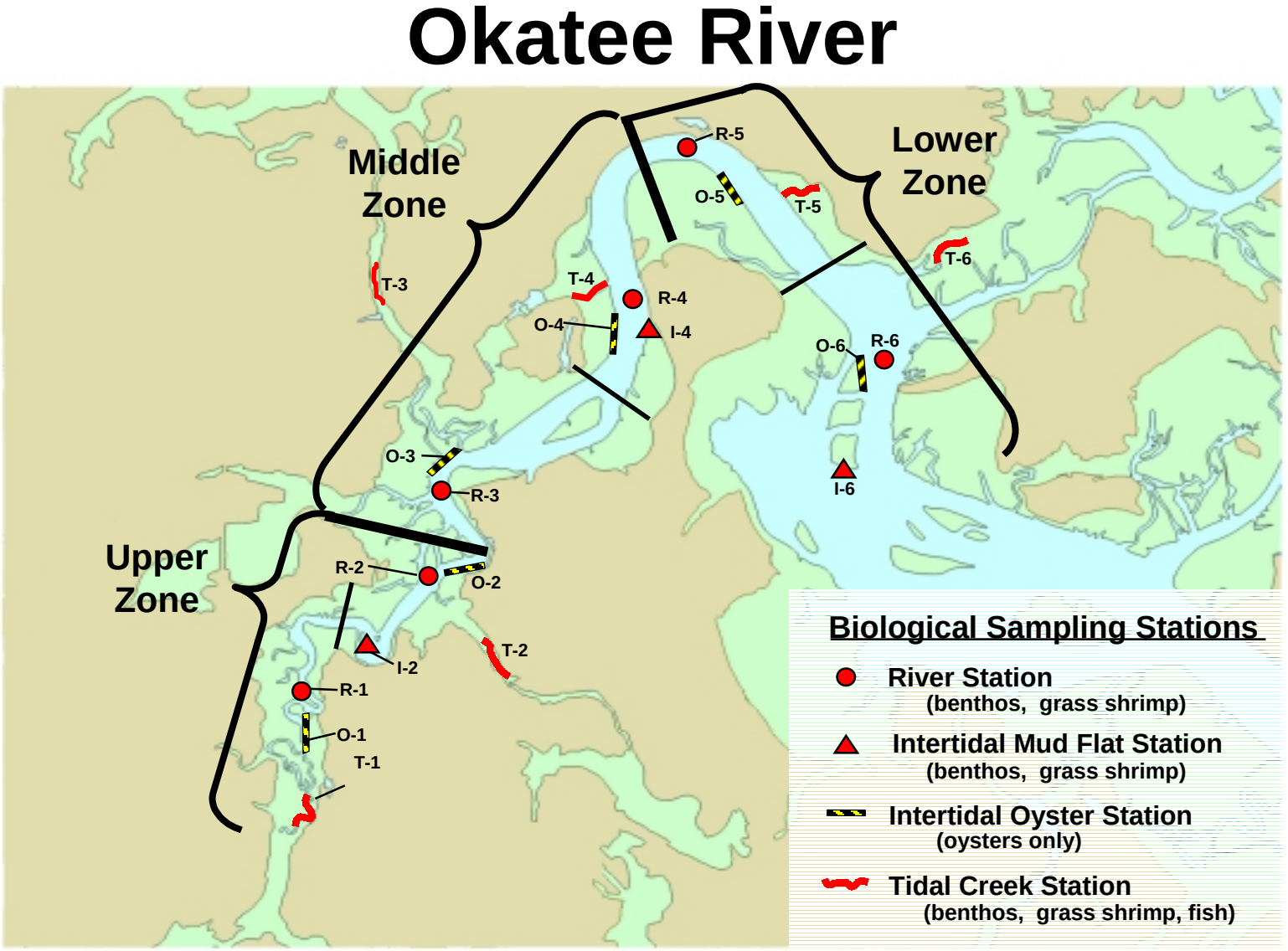


Figure 5.2. Map of Okatee River stations sampled for biota in 1998.

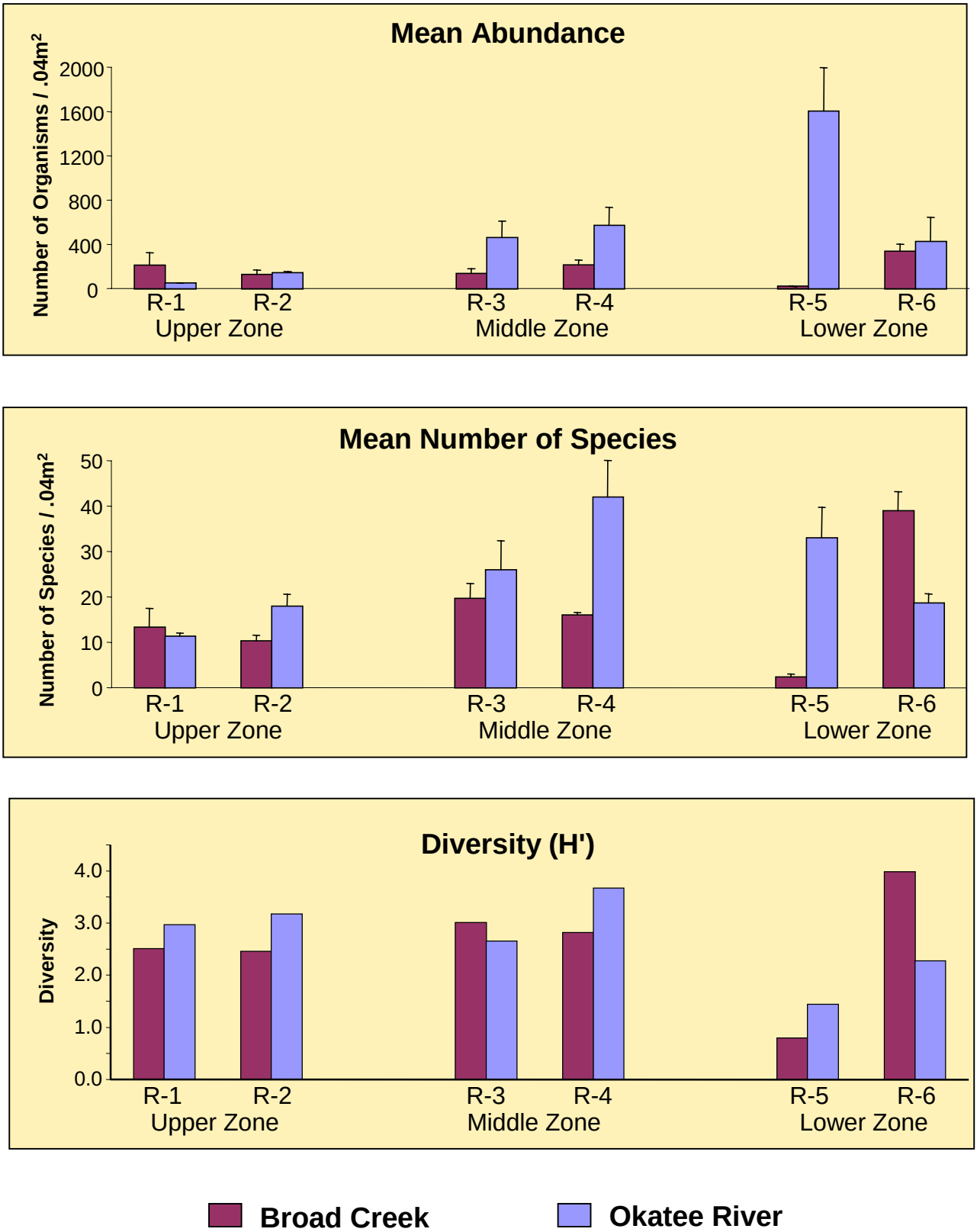


Figure 5.3. Summary of the mean abundance, mean number of species, and species diversity from subtidal river stations in Broad Creek and the Okatee River. Error bars represent 1 standard error.

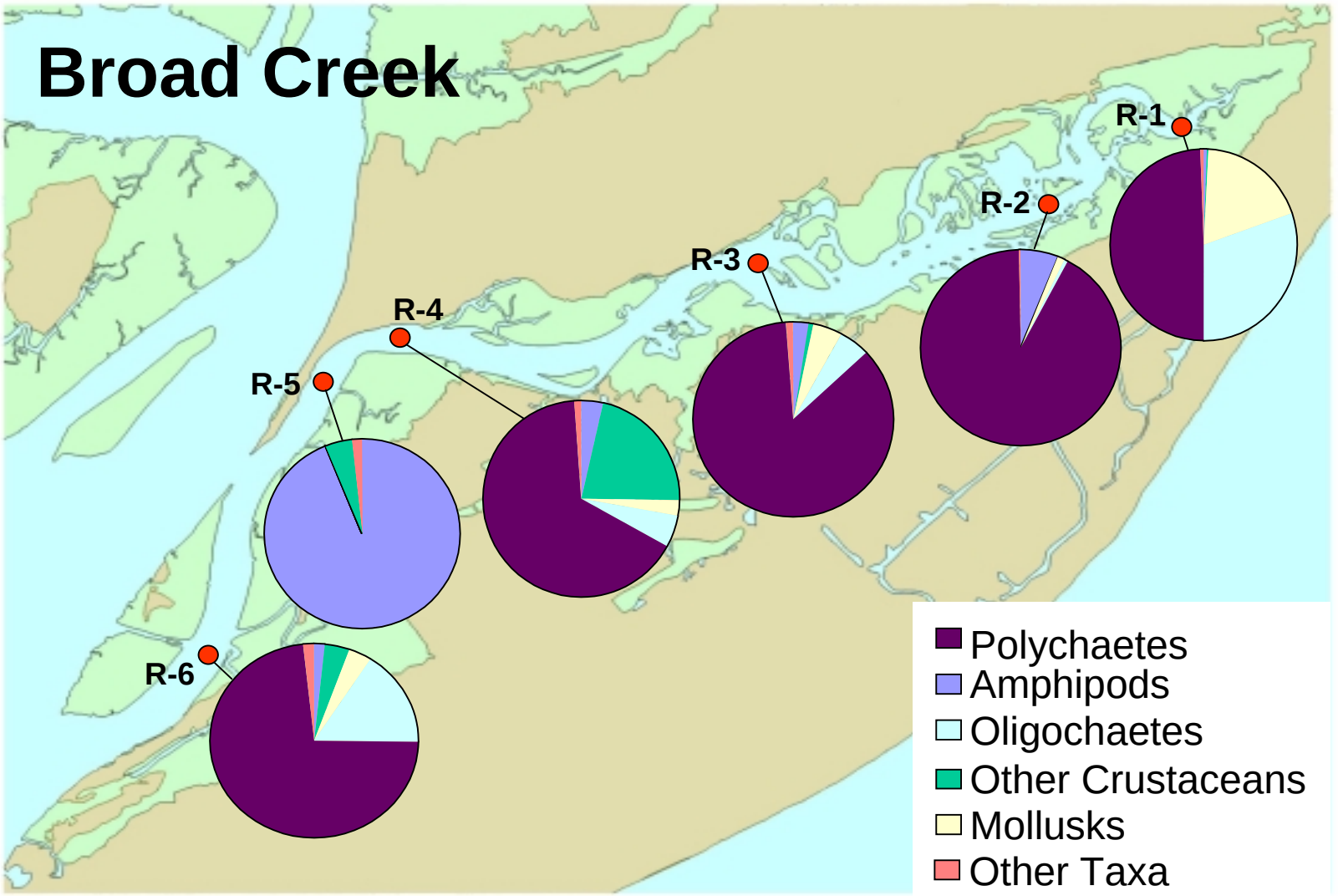


Figure 5.4. Composition of the benthic macrofauna collected from subtidal stations in Broad Creek

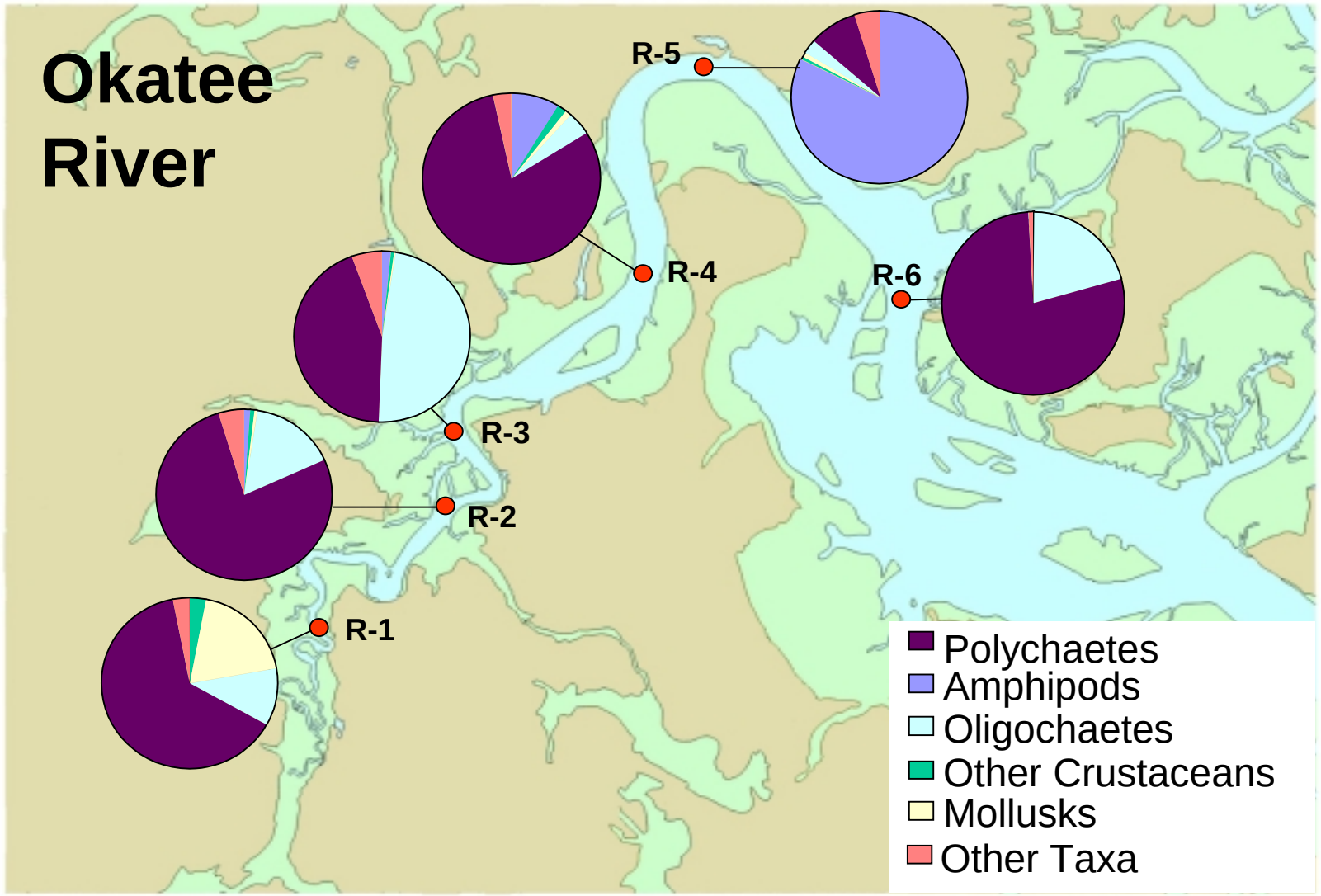


Figure 5.5. Composition of benthic macrofauna collected from subtidal stations in the Okatee River.

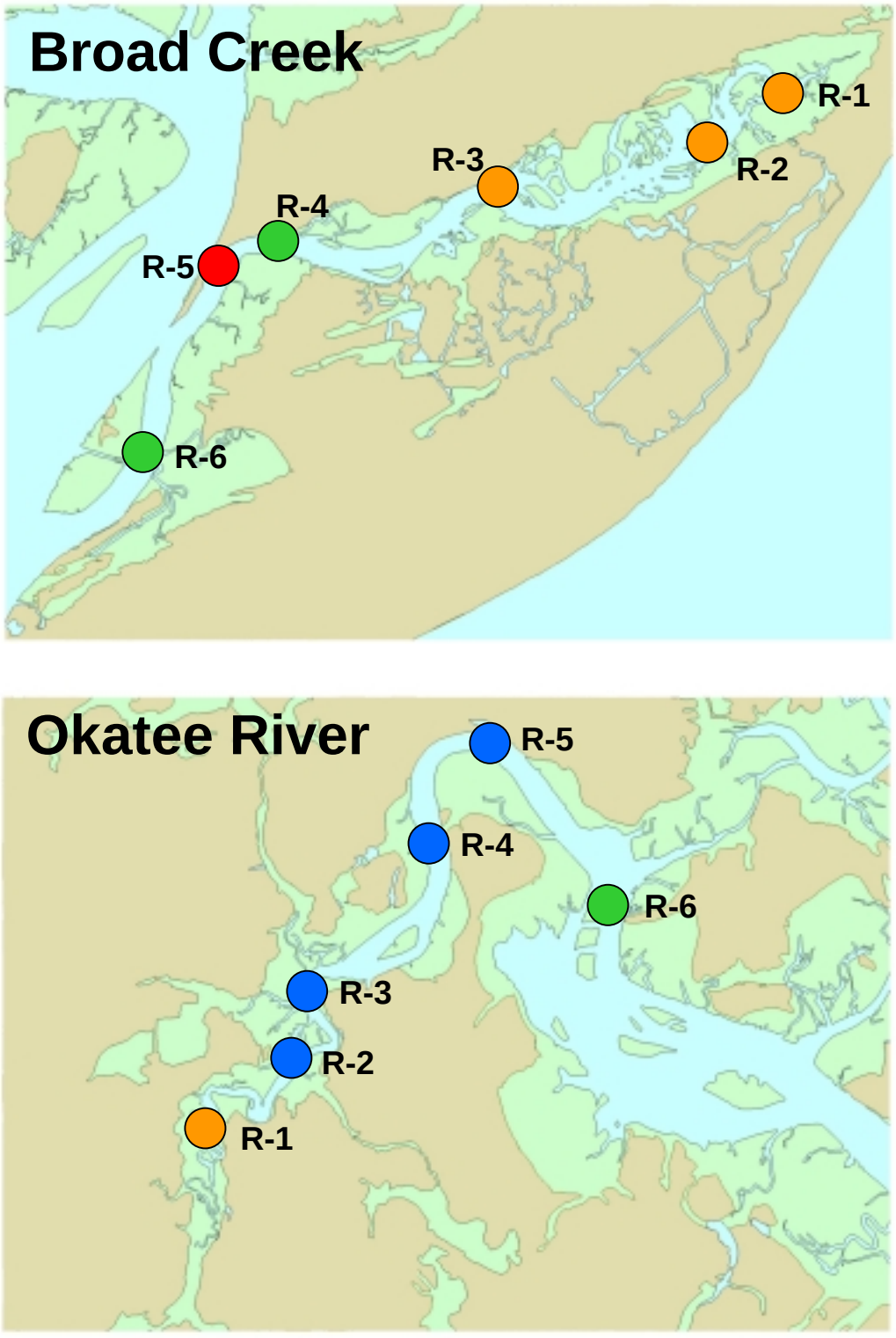


Figure 5.6. Subtidal benthic stations groups which showed greater similarity in faunal composition among sites (same color) than with other site groups (different colors).

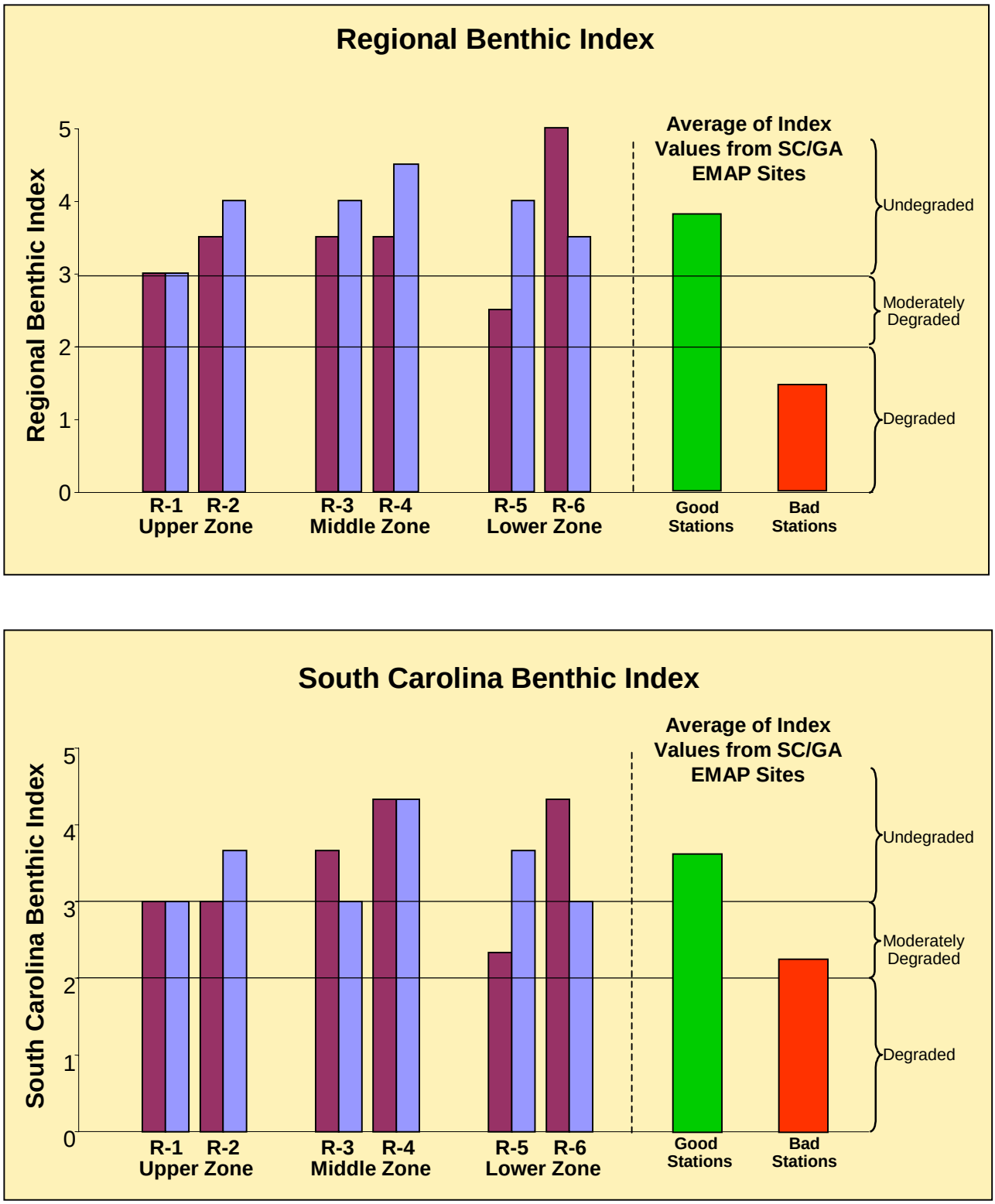
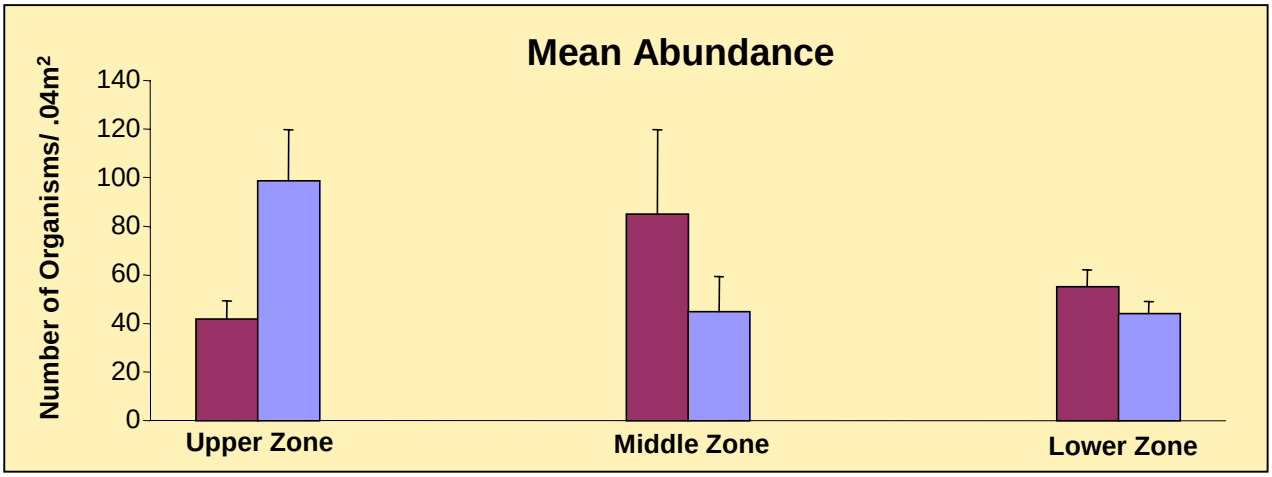
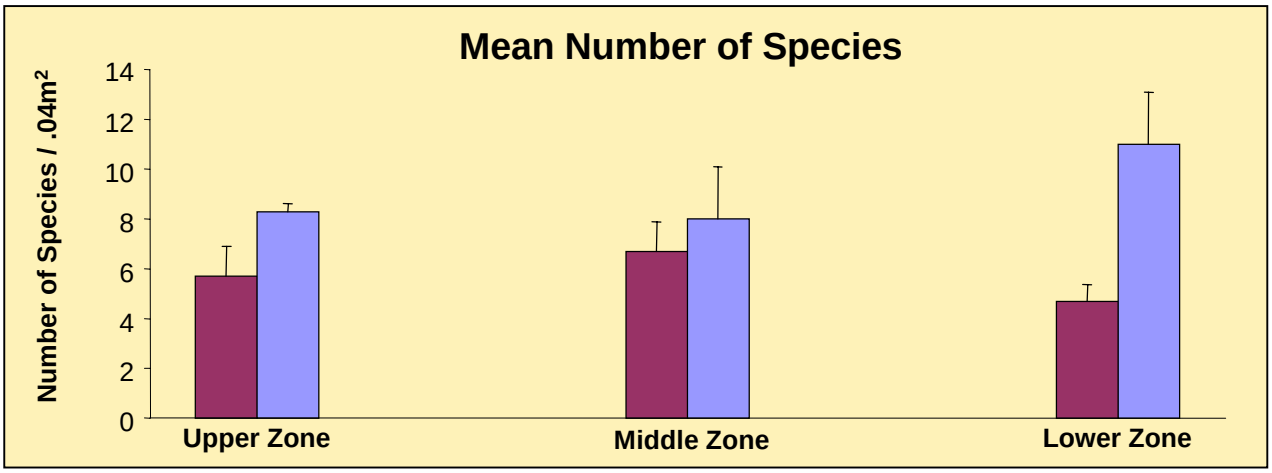
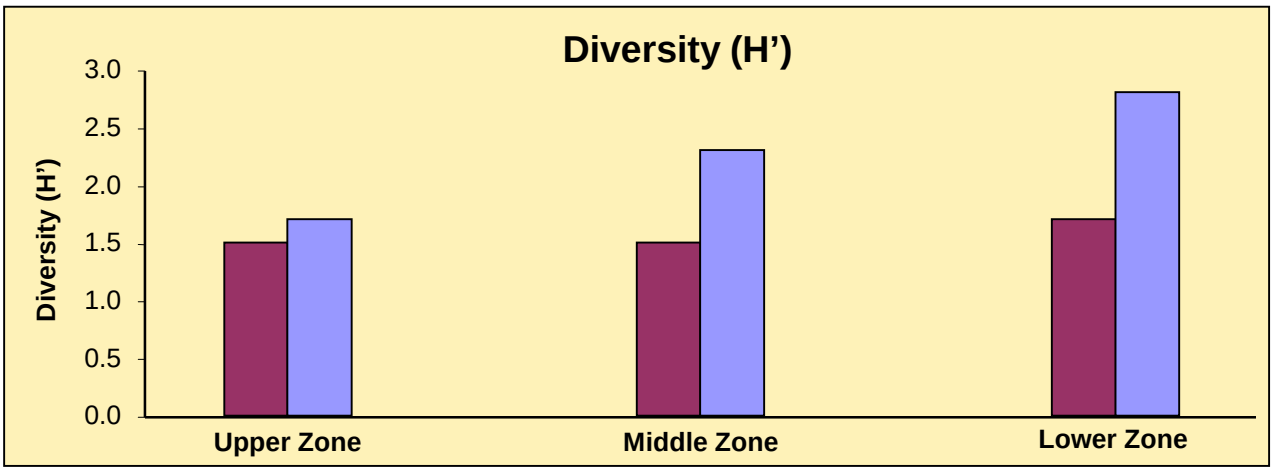


Figure 5.7. Benthic Index of Biological Integrity (B-IBI) results for subtidal river stations sampled in Broad Creek and the Okatee River.







Broad Creek Okatee River

Figure 5.8. Summary of the mean abundance, mean number of species, and species diversity at intertidal mud flats in Broad Creek and the Okatee River. Error bars represent 1 standard error.

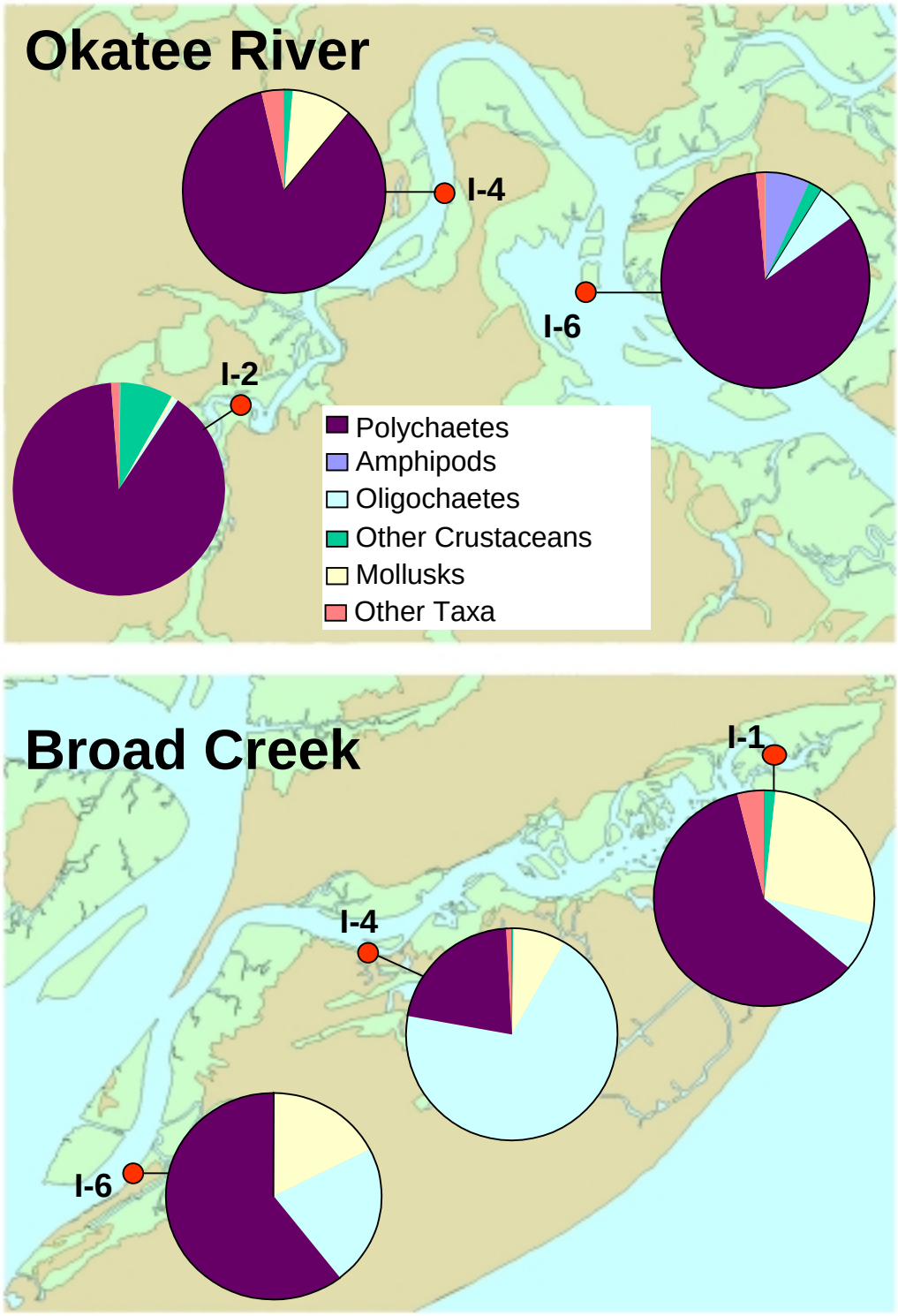


Figure 5.9. Composition of benthic macrofauna collected from intertidal mud flats in Broad Creek and the Okatee River.

Overall Summary, By River

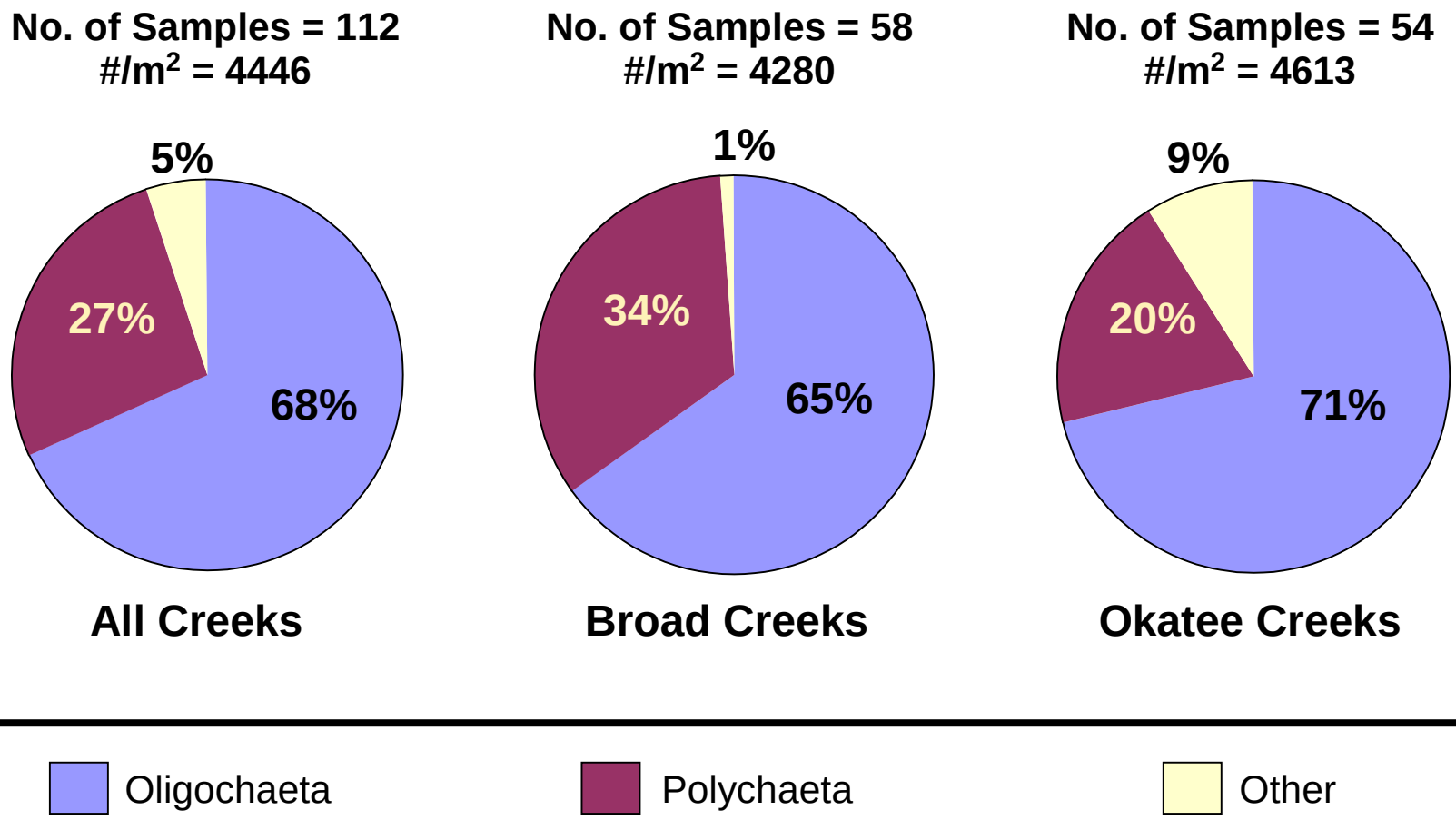
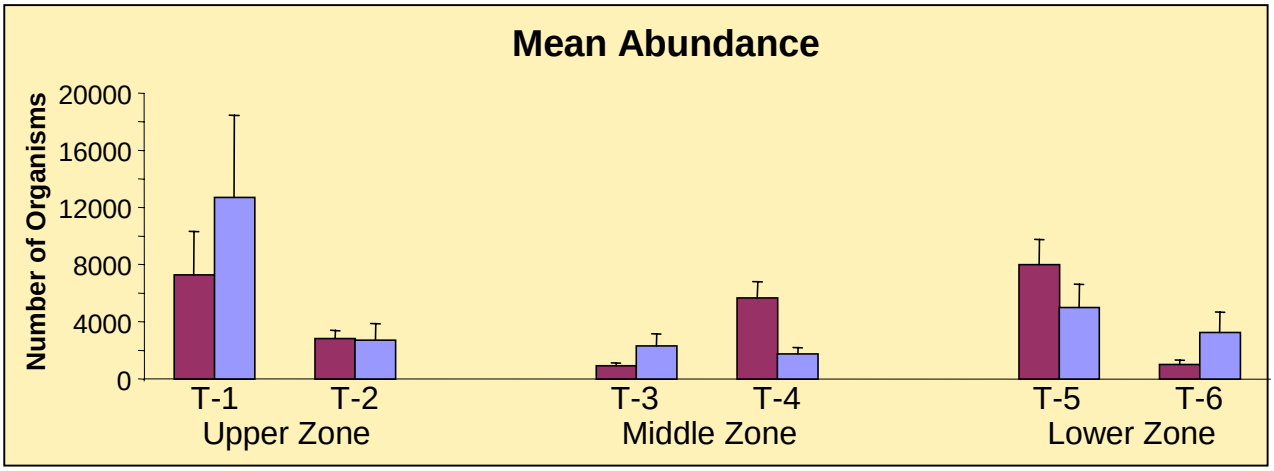
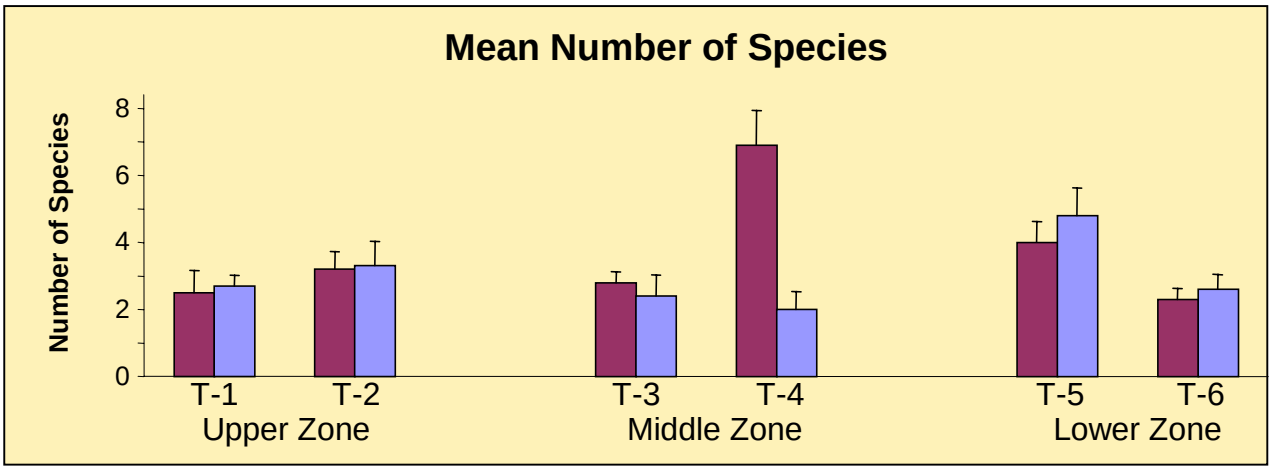


Figure 5.10. Taxonomic composition of benthic communities in tidal creeks of Broad Creek and the Okatee River.





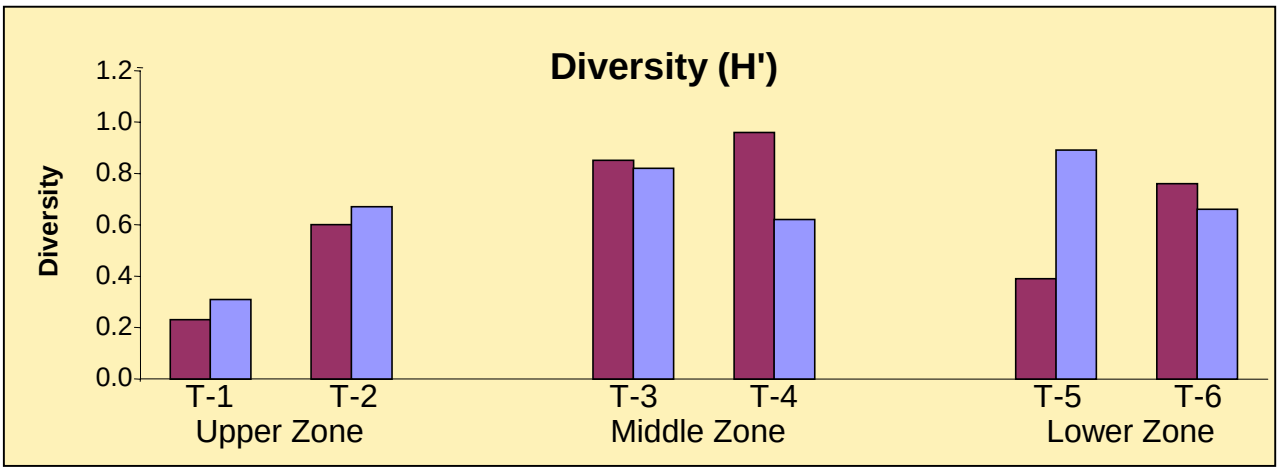


Figure 5.11. Summary of the mean abundance, mean number of species, and species diversity from tidal creeks in Broad Creek and the Okatee River. Error bars represent 1 standard error.

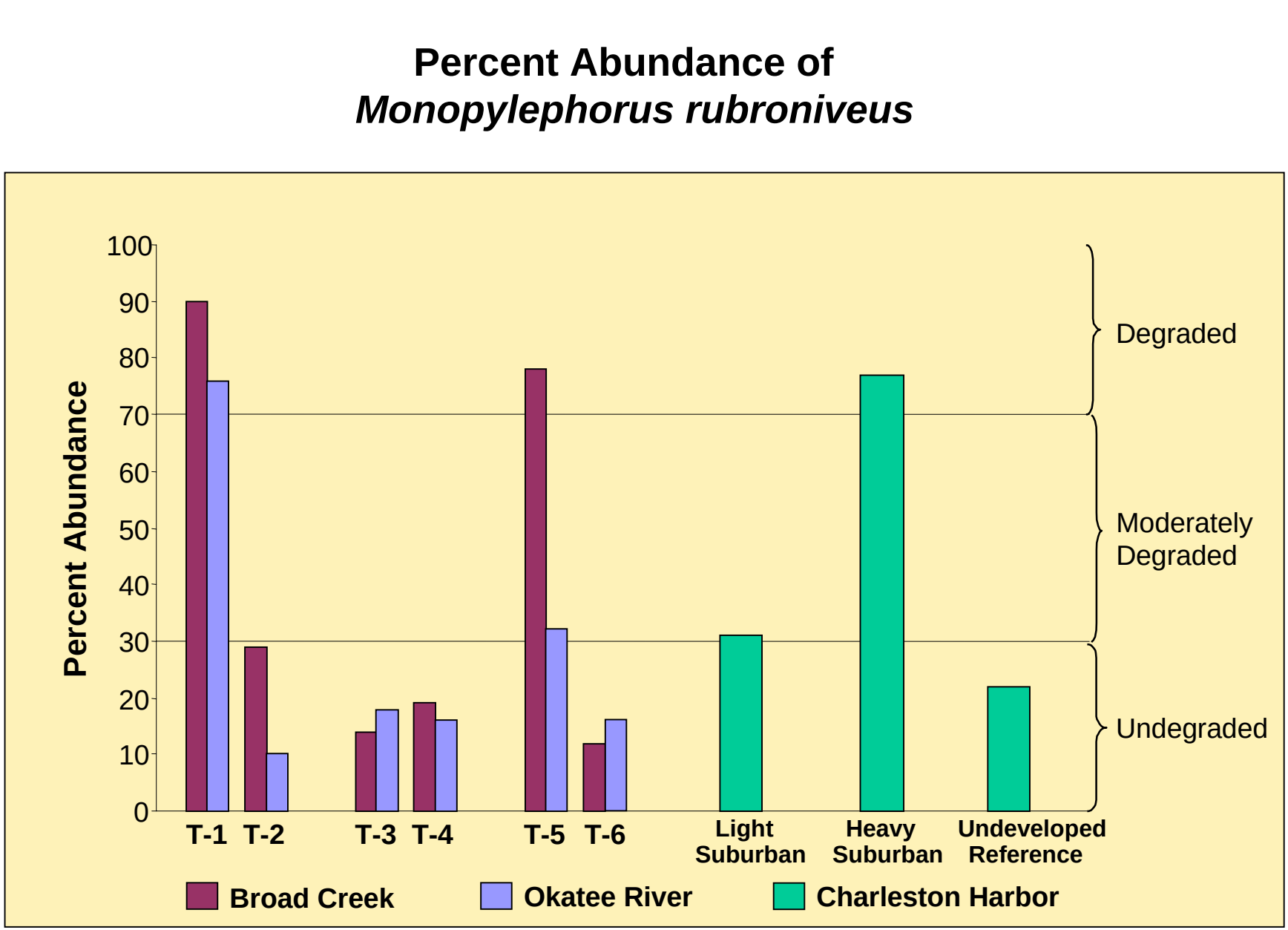


Figure 5.12. Habitat quality results for tidal creeks in Broad Creek and the Okatee River, based on the percent abundance of the oligochaete *Monopylephorus rubroniveus*.

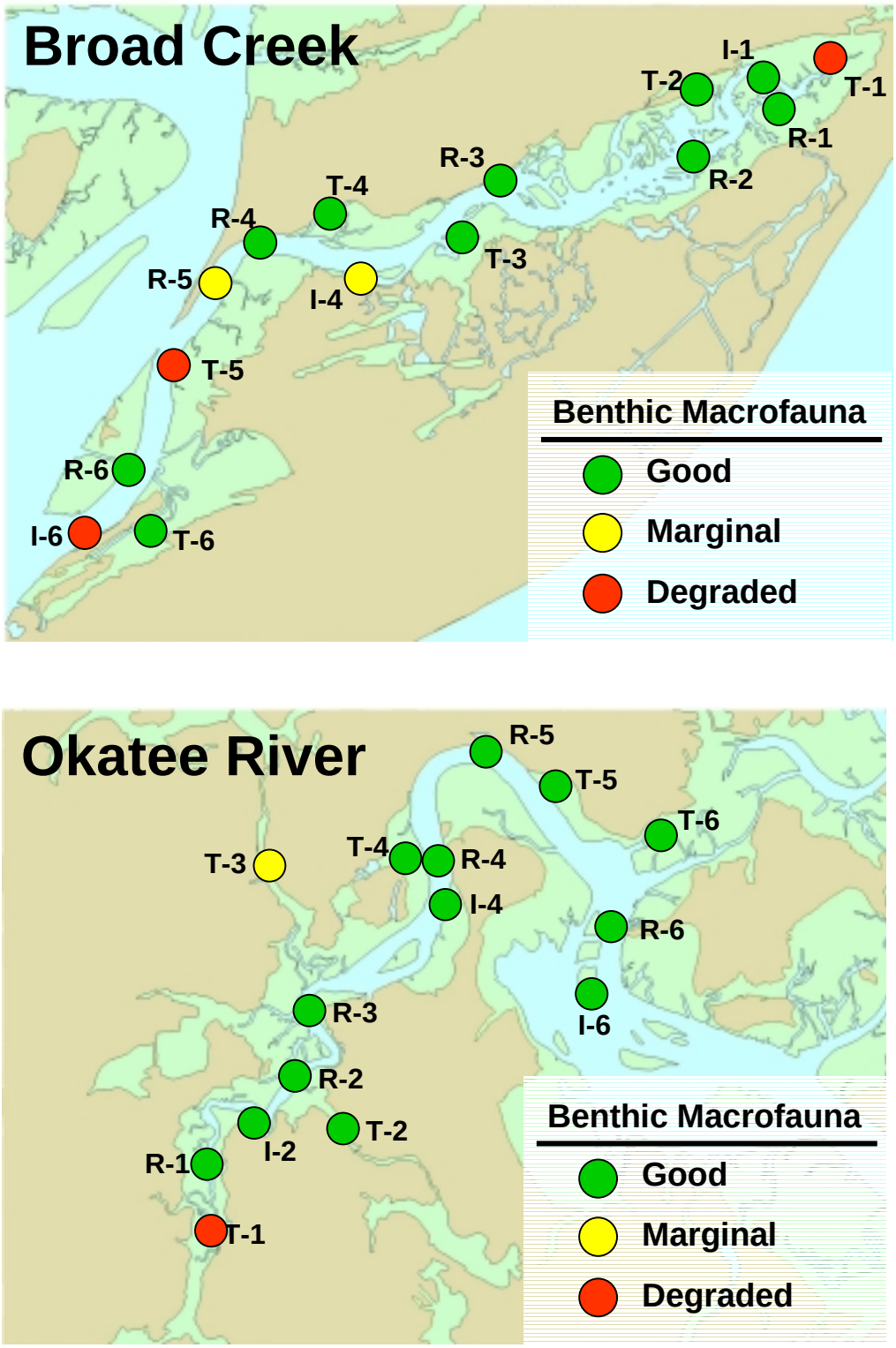


Figure 5.13. Summary of the condition of benthic macrofauna in Broad Creek and the Okatee River.

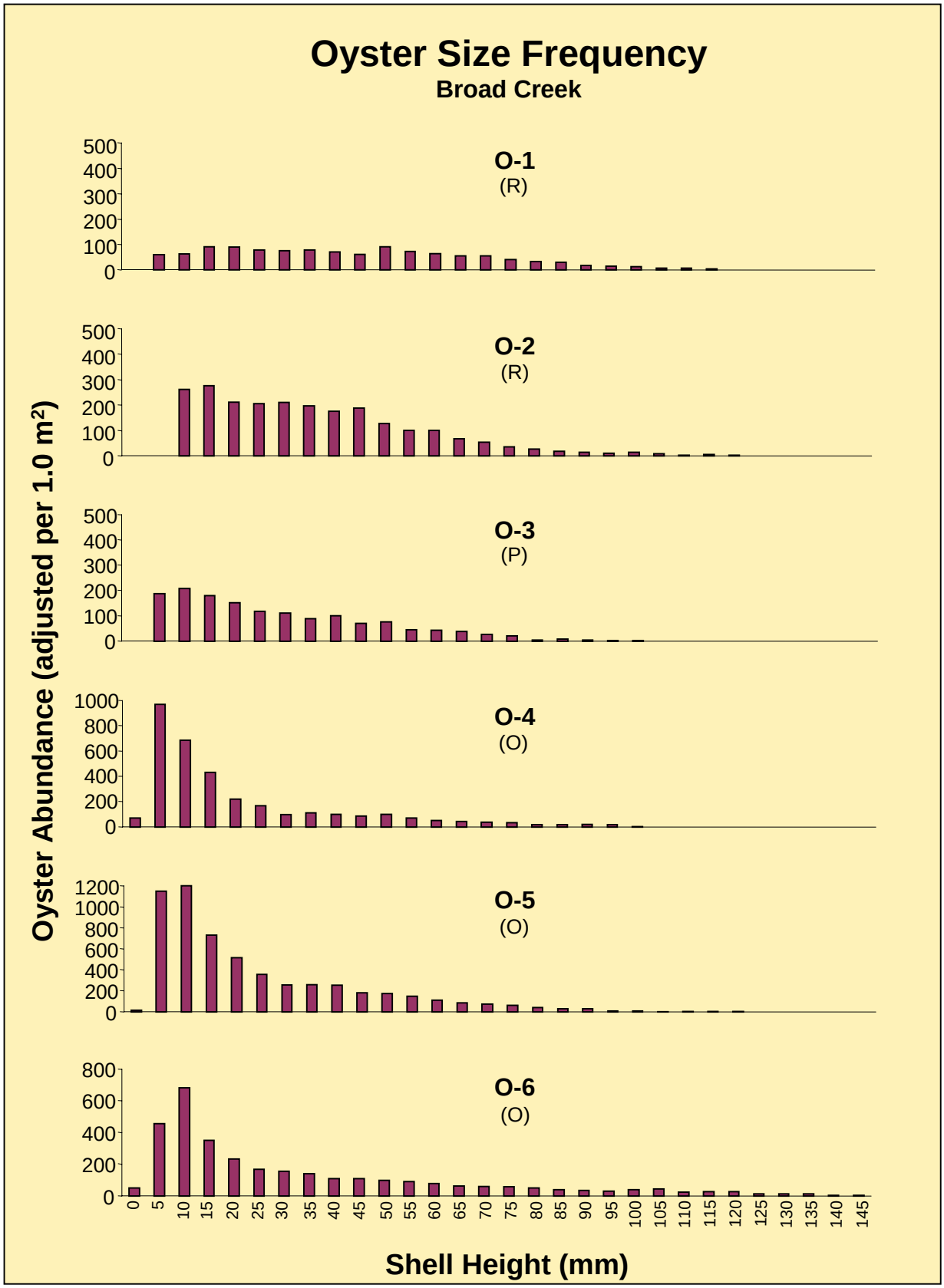


Figure 5.14. Size frequency distribution of live oysters collected in Broad Creek. (R) = Restricted, (O) = Open, (P) = Prohibited.

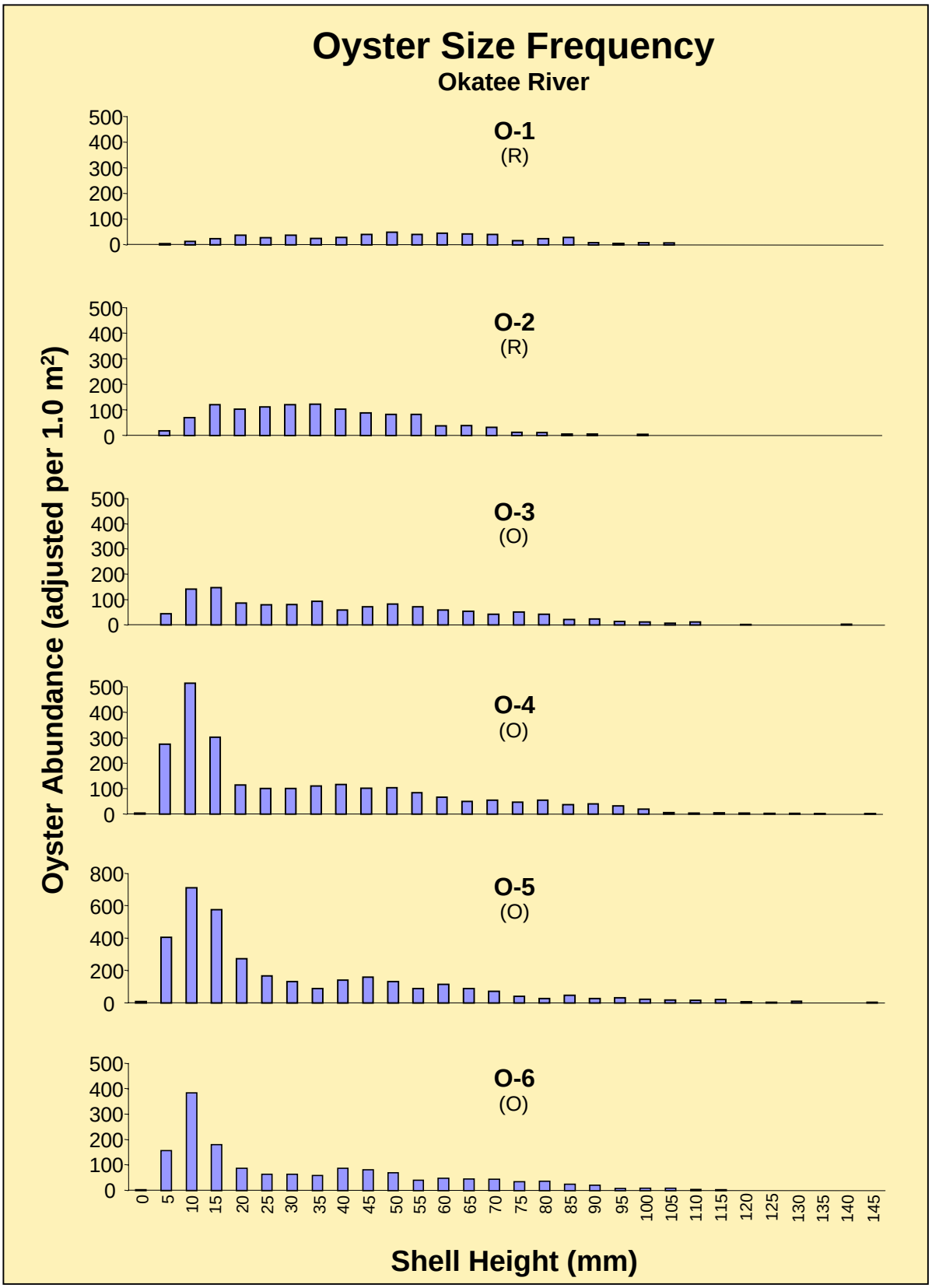


Figure 5.15. Size frequency distribution of live oysters collected in the Okatee River. (R) = Restricted; (O) = Open.

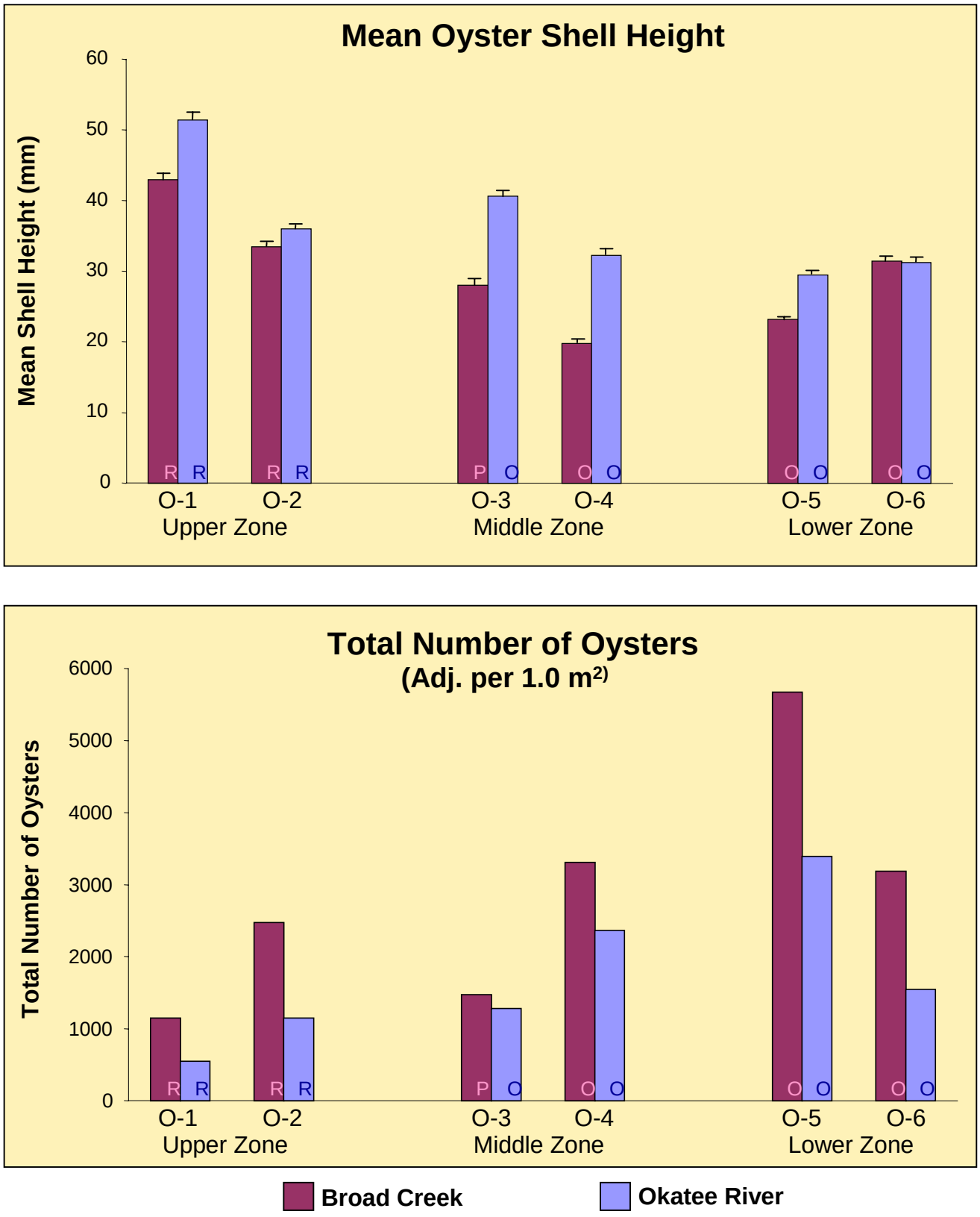


Figure 5.16. Mean oyster shell heights and total number of oysters (summed over 5 samples) at 12 stations in Broad Creek and the Okatee River. R = Restricted; P = Prohibited; O = Open. Error bars represent 1 standard error.

Dermo Prevalence and Intensity

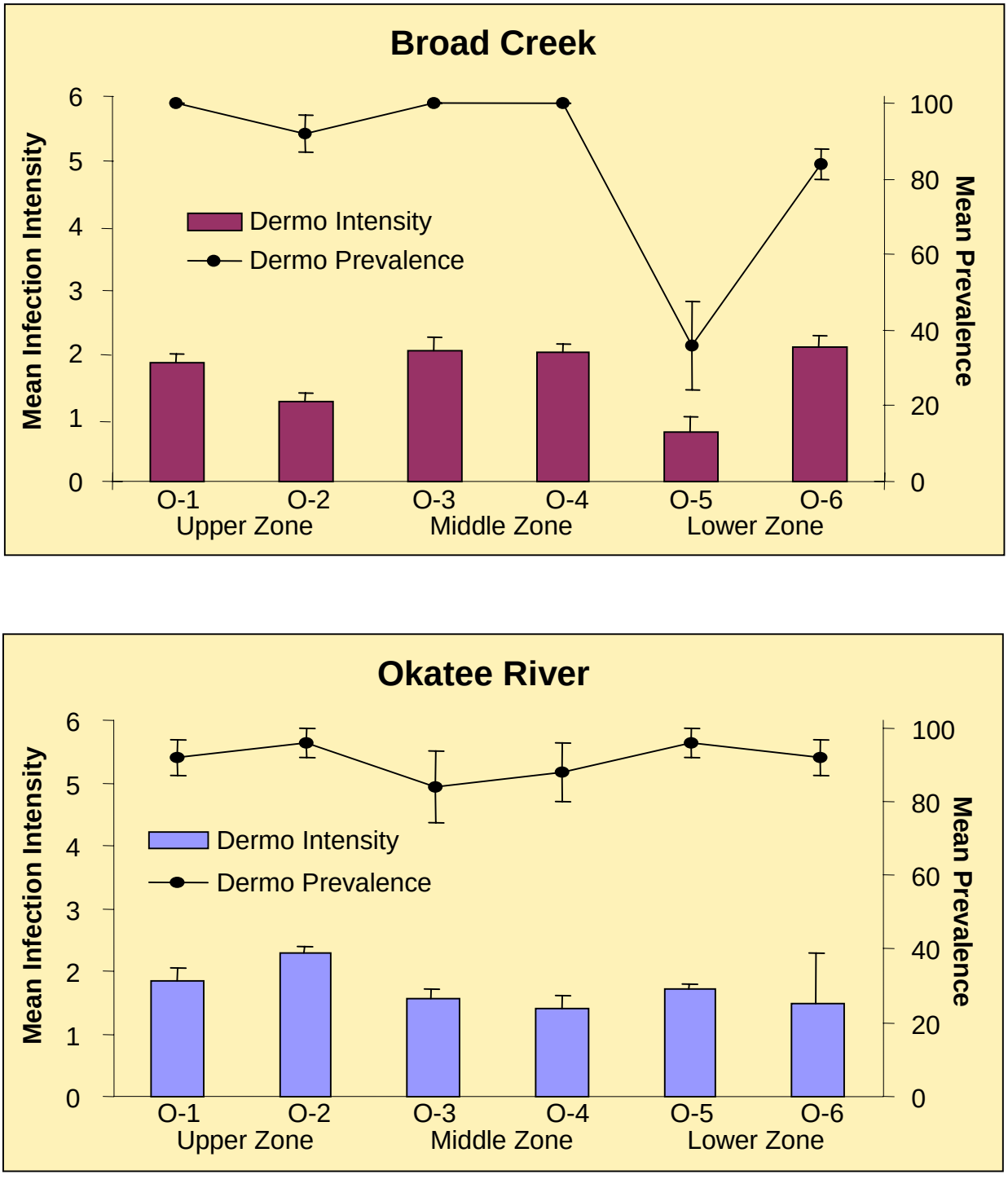


Figure 5.17. The prevalence and intensity of Dermo in oysters sampled in Broad Creek and the Okatee River. Error bars represent 1 standard error.

Prevalence of MSX in Oysters

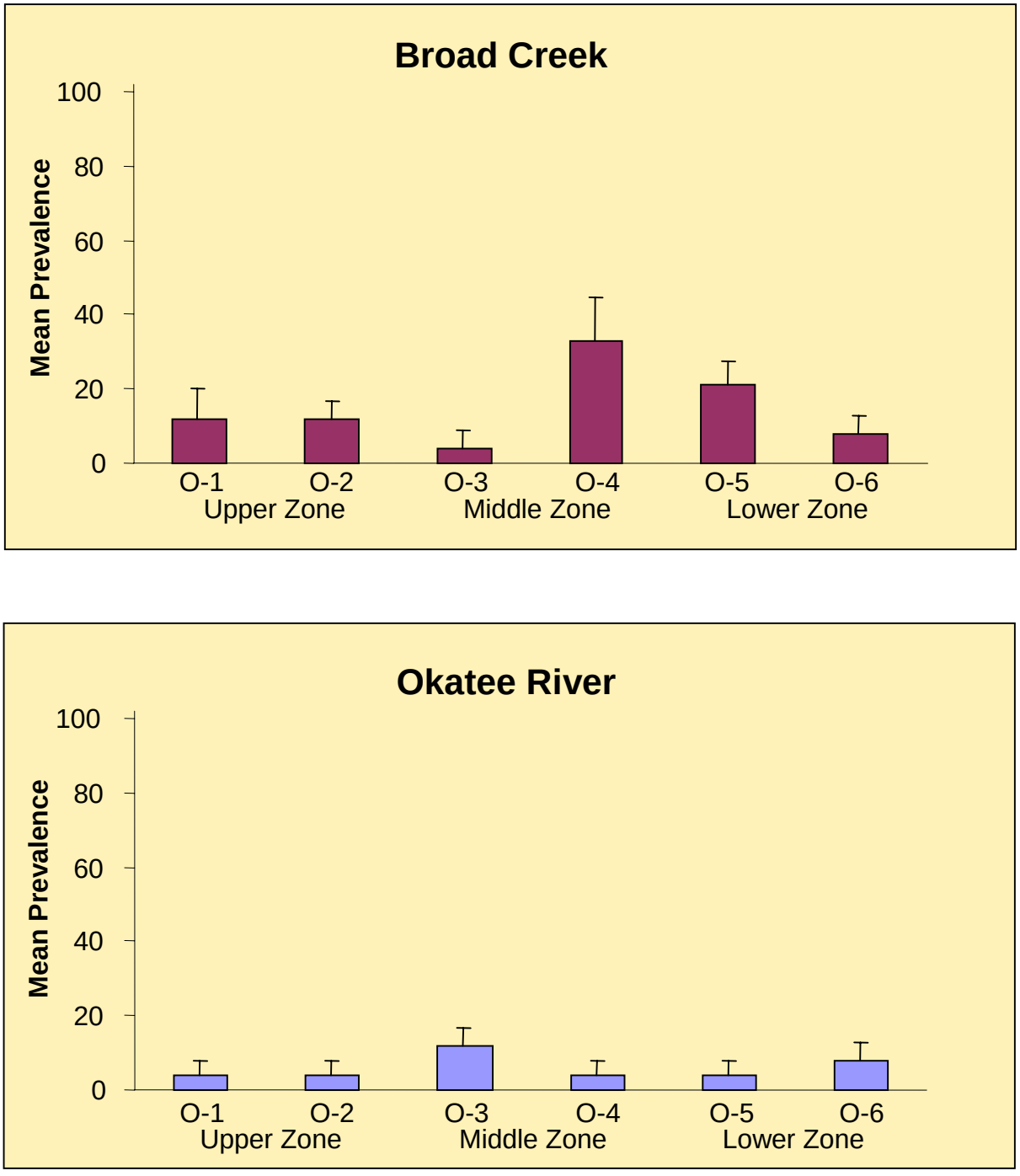


Figure 5.18. Prevalence of MSX in oysters sampled in Broad Creek and the Okatee River during summer 1997. Error bars represent 1 standard error.

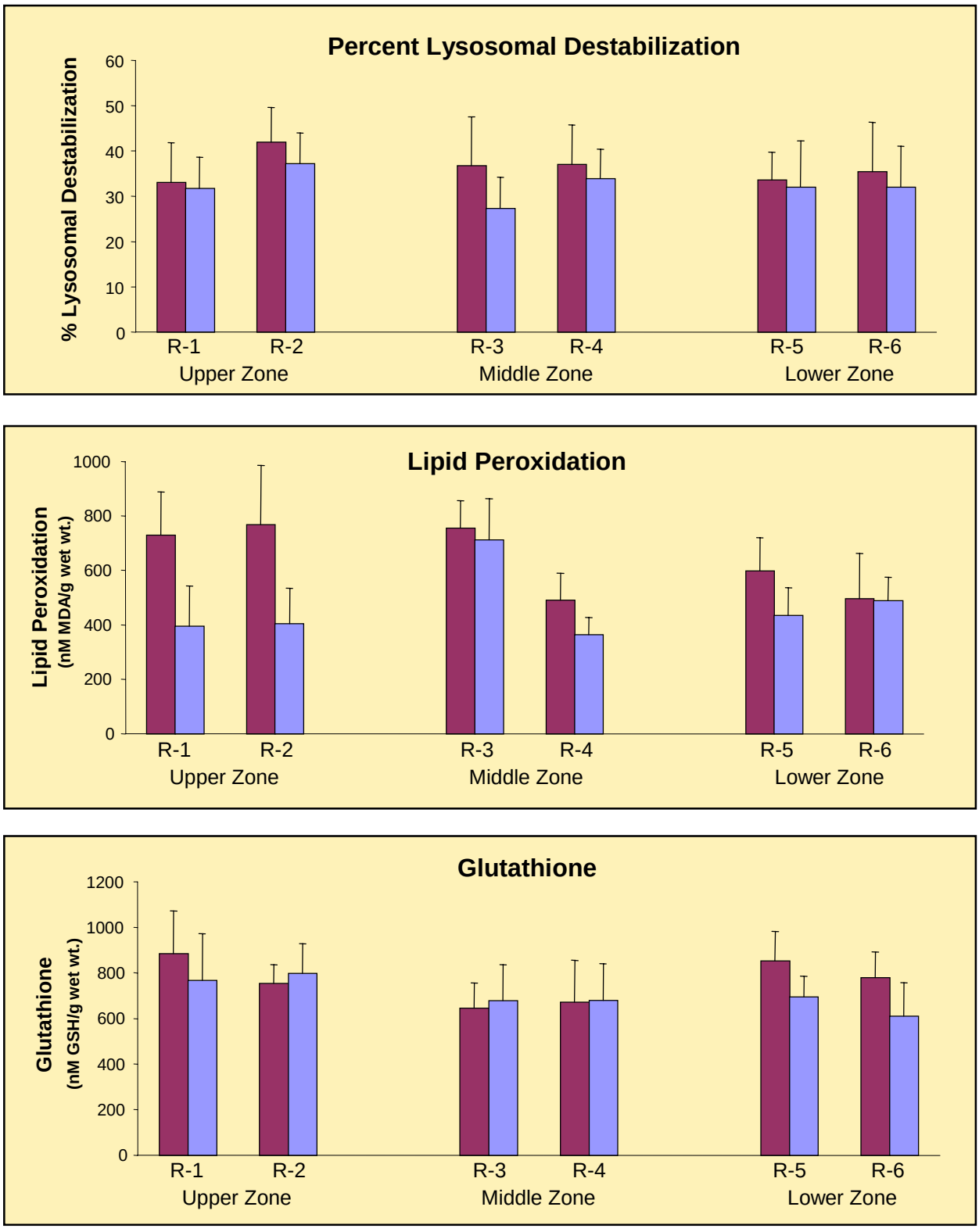


Figure 5.19. Cellular responses of oysters collected from Broad Creek and the Okatee River. A) Percent lysosomal destabilization; B) Lipid Peroxidation; C) Glutathione concentrations. Error bars represent 1 standard deviation.

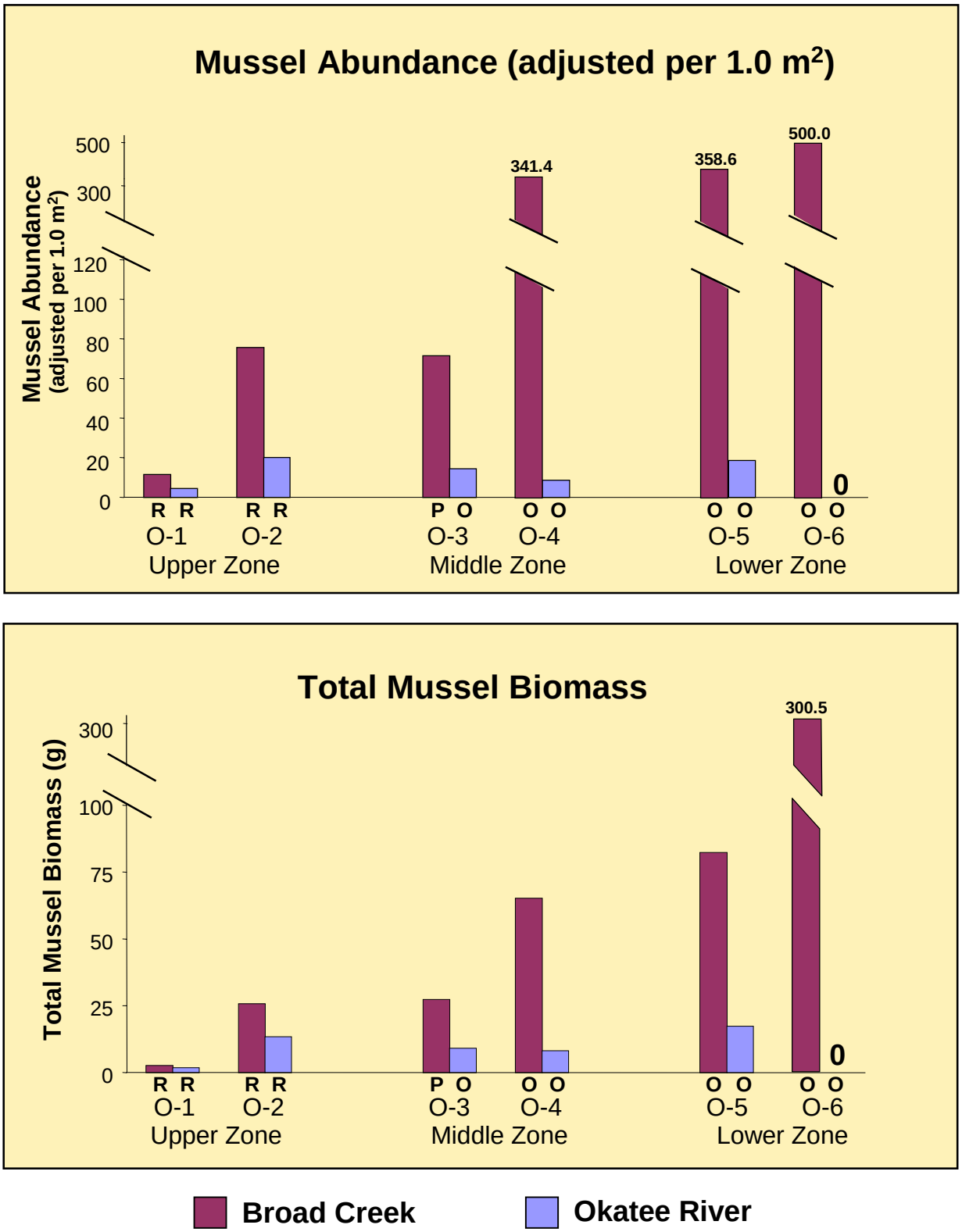


Figure 5.20. Mean mussel biomass at stations sampled in Broad Creek and the Okatee River. R = Restricted; P = Prohibited; O = Open.

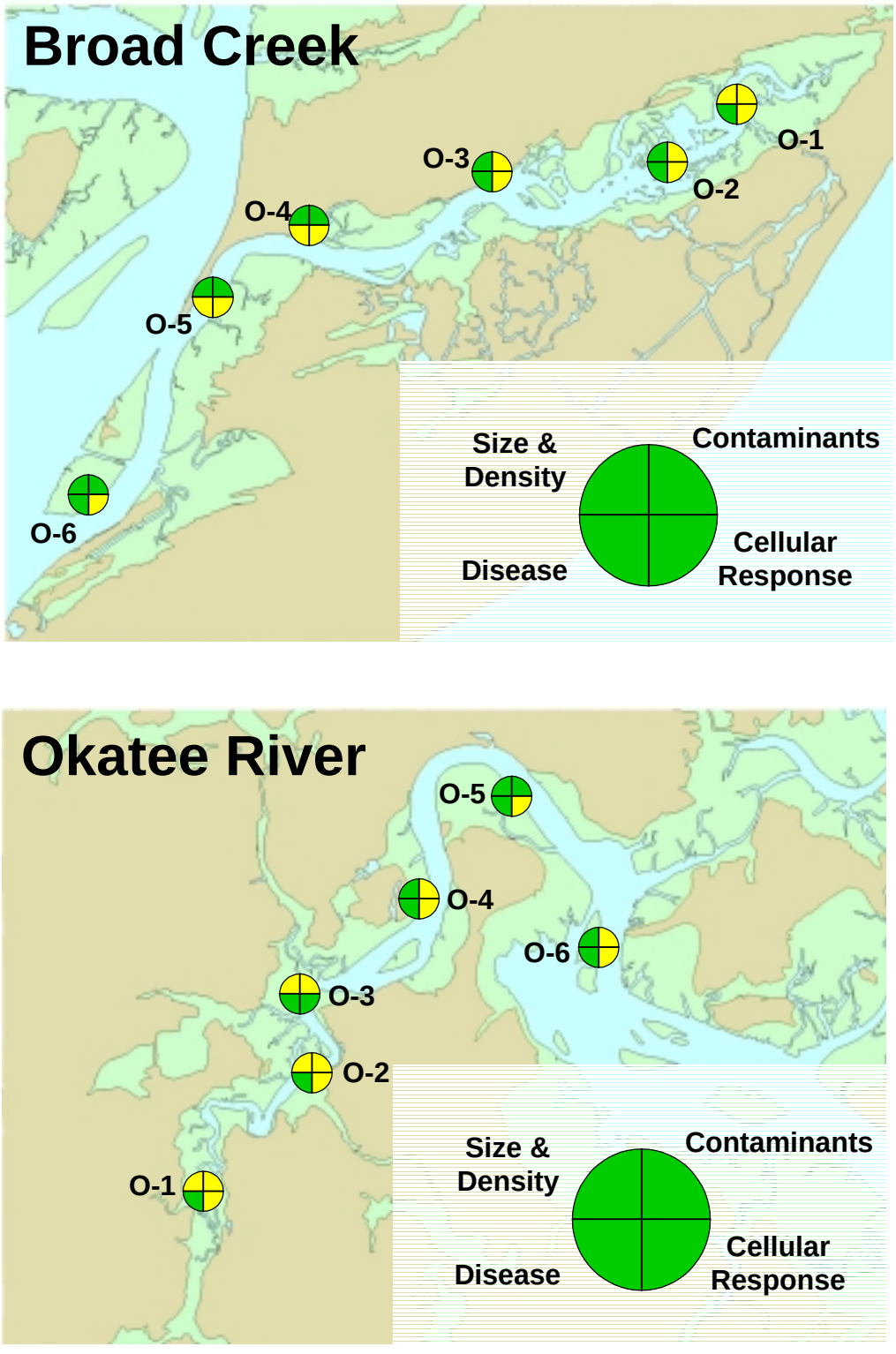


Figure 5.21. Summary of oyster bed condition in Broad Creek and the Okatee River.

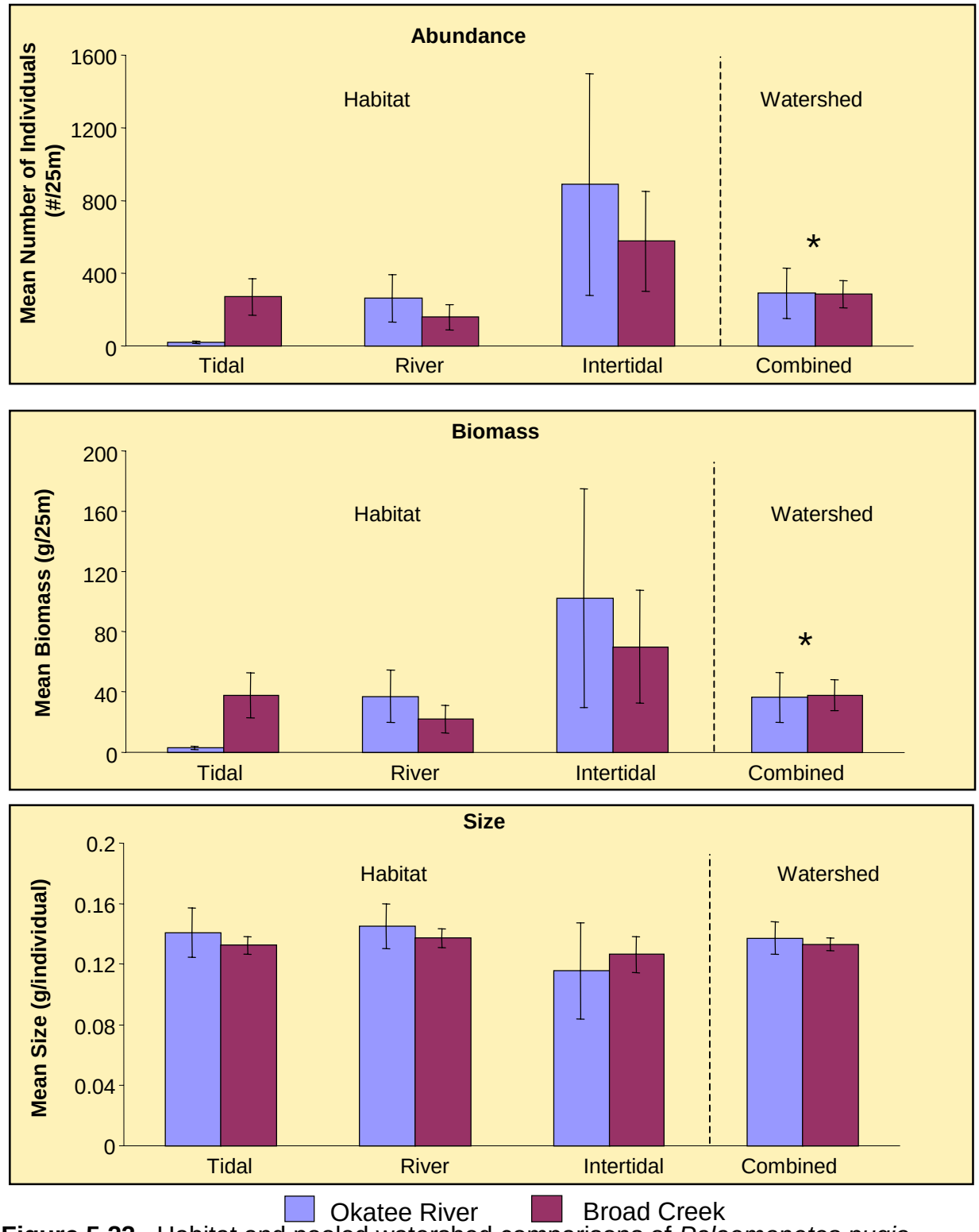


Figure 5.22. Habitat and pooled watershed comparisons of *Palaemonetes pugio* abundance, biomass and size. An asterisk indicates significant ($p<0.05$) difference. Error bars represent $\pm$1 standard error.

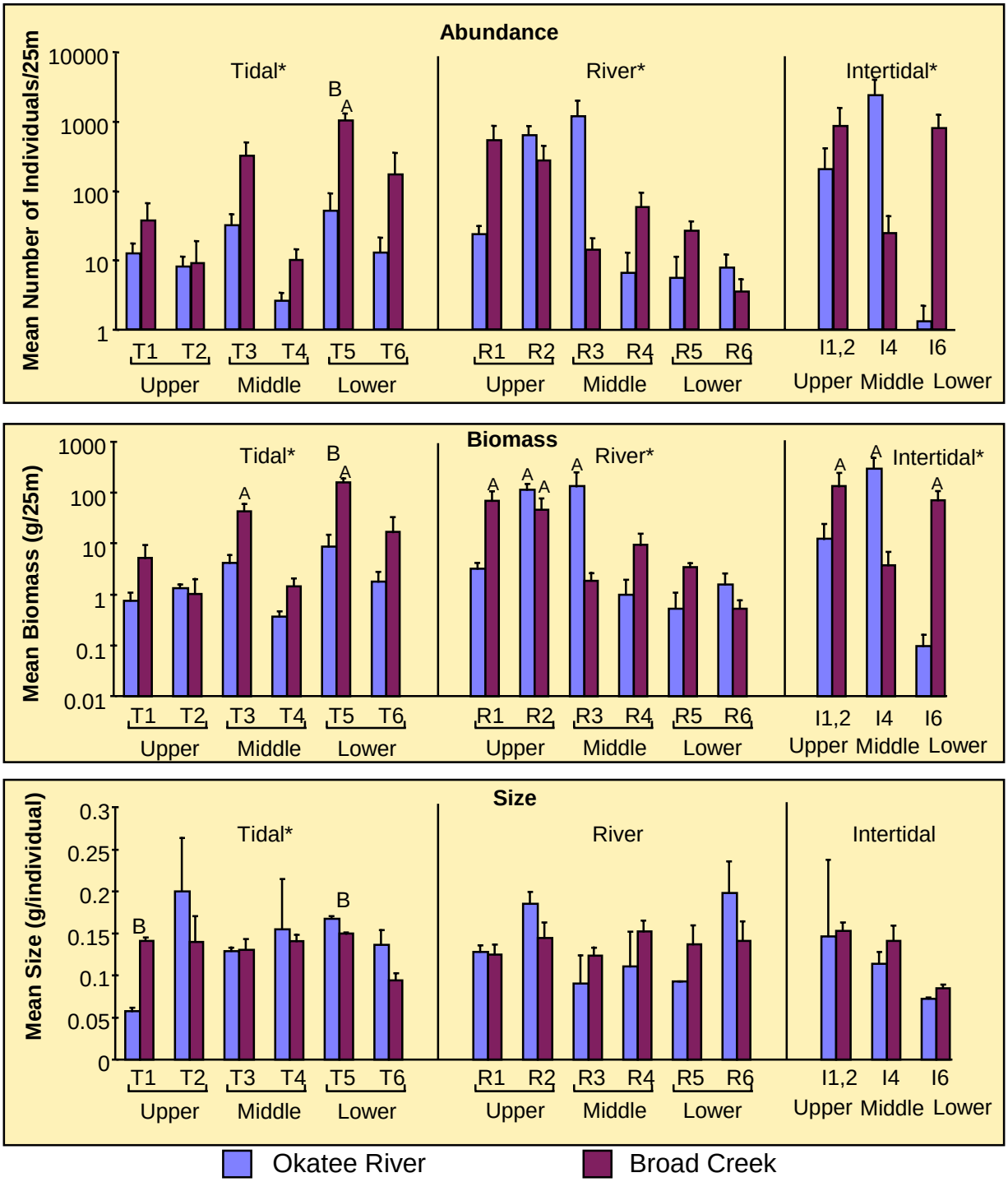


Figure 5.23. Station by station comparisons of *Palaemonetes pugio* abundance, biomass and size. An asterisk indicates significant ($p<0.05$) difference by habitat, "A" indicates significantly ($p<0.05$) greater than 1 or more other sites, and "B" indicates significantly ($p<0.05$) different between systems by corresponding sites. Error bars represent 1 standard error.

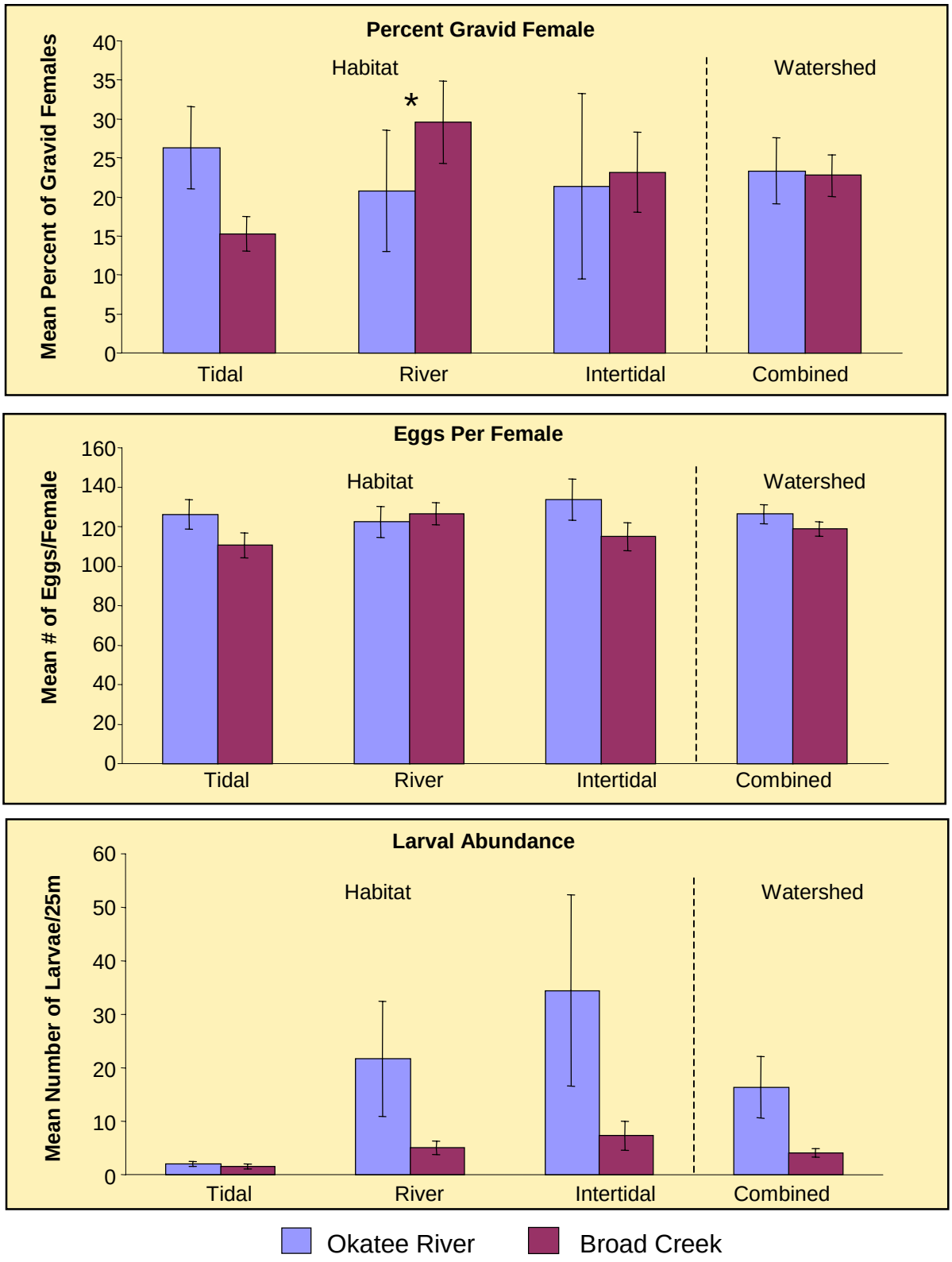


Figure 5.24. Habitat and pooled watershed comparisons of *Palaemonetes pugio* percent gravid females, eggs per female, and larval abundance. An asterisk indicates significant ($p<0.05$) difference. Error bars represent $\pm$1 standard error.

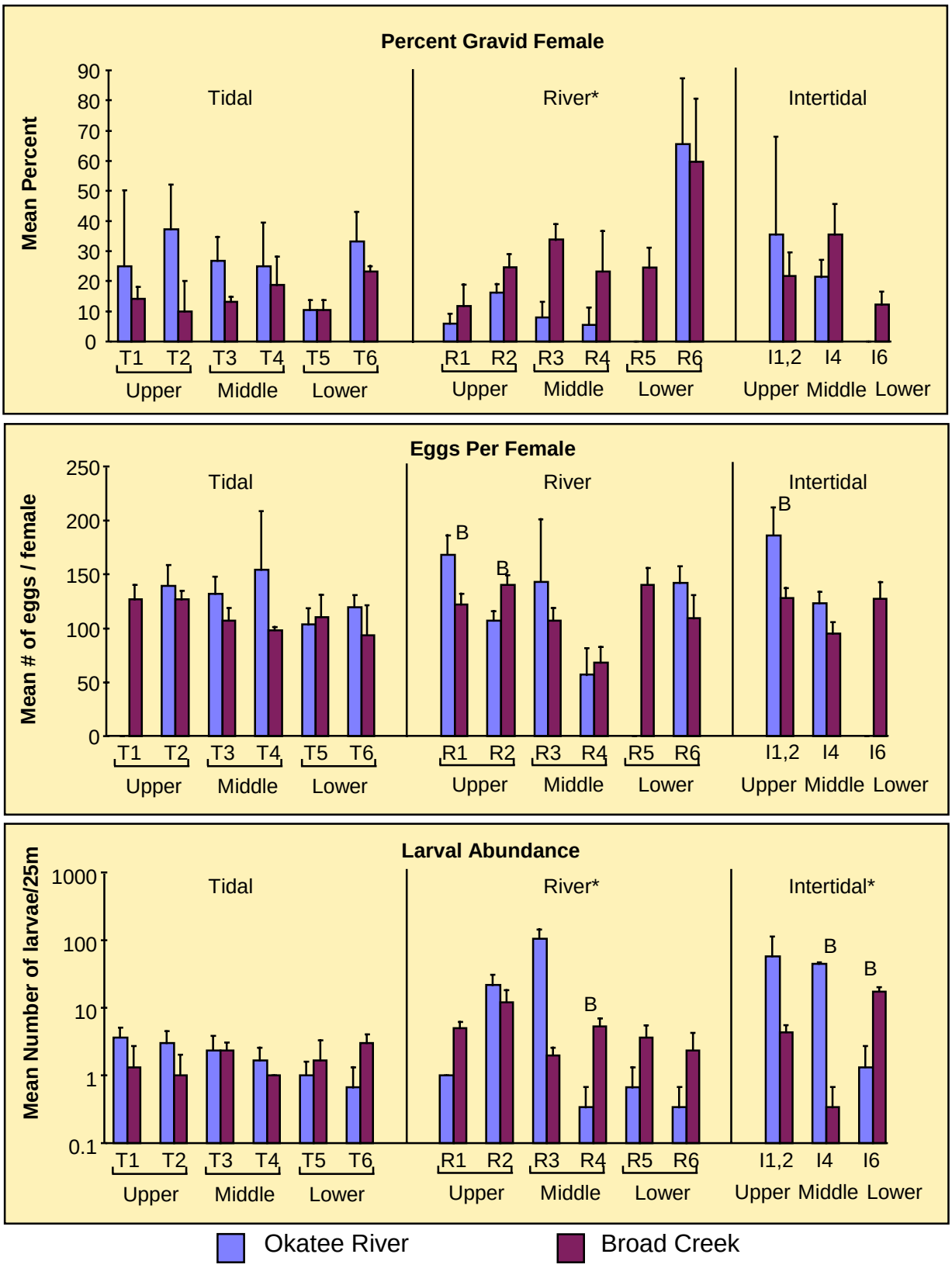


Figure 5.25. Station by station comparisons of *Palaemonetes pugio* percent gravid females, eggs per female and larval abundance. An asterisk indicates significant ($p<0.05$) difference by habitat, “A” indicates significantly ($p<0.05$) greater than 1 or more other sites, and “B” indicates significantly ($p<0.05$) different between systems by corresponding sites. Error bars represent 1 standard error.

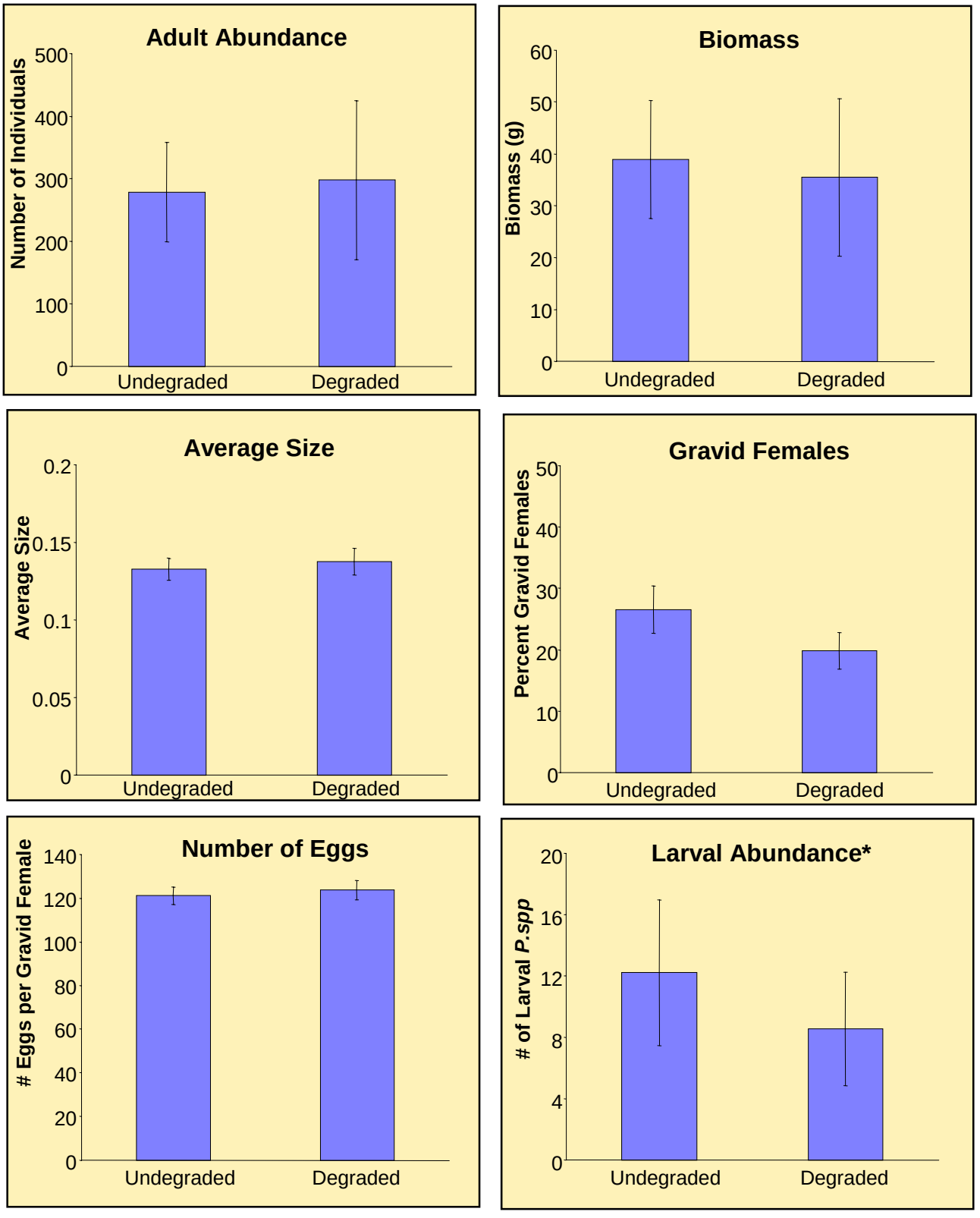


Figure 5.26. Comparison of *Palaemonetes pugio* population metrics at degraded and undegraded sites. An asterisk indicates significant ($p<0.05$) difference. Error bars represent $\pm$1 standard error.

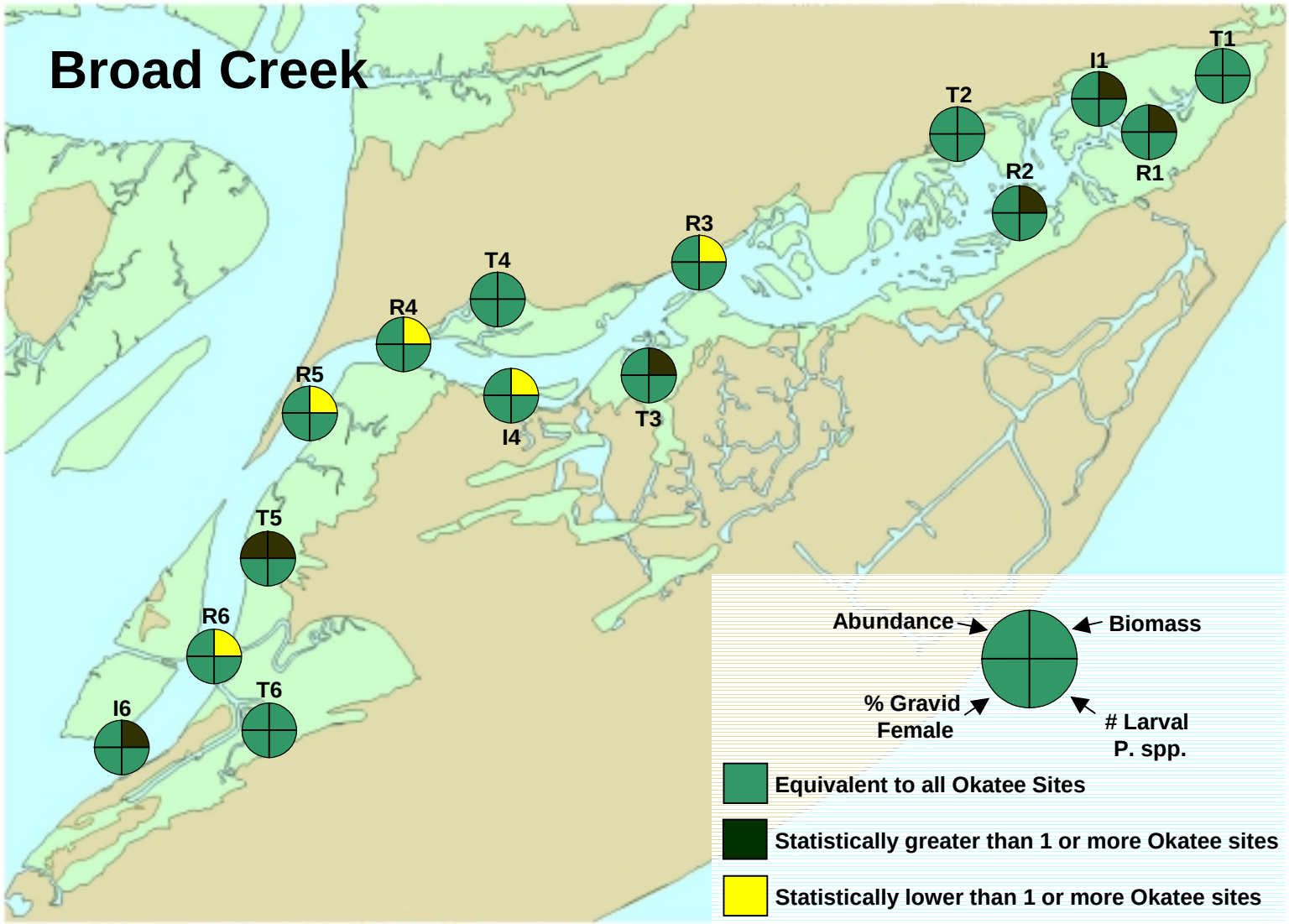


Figure 5.27. Map of Broad Creek showing *Palaemonetes spp*. population metrics relative to the Okatee River.

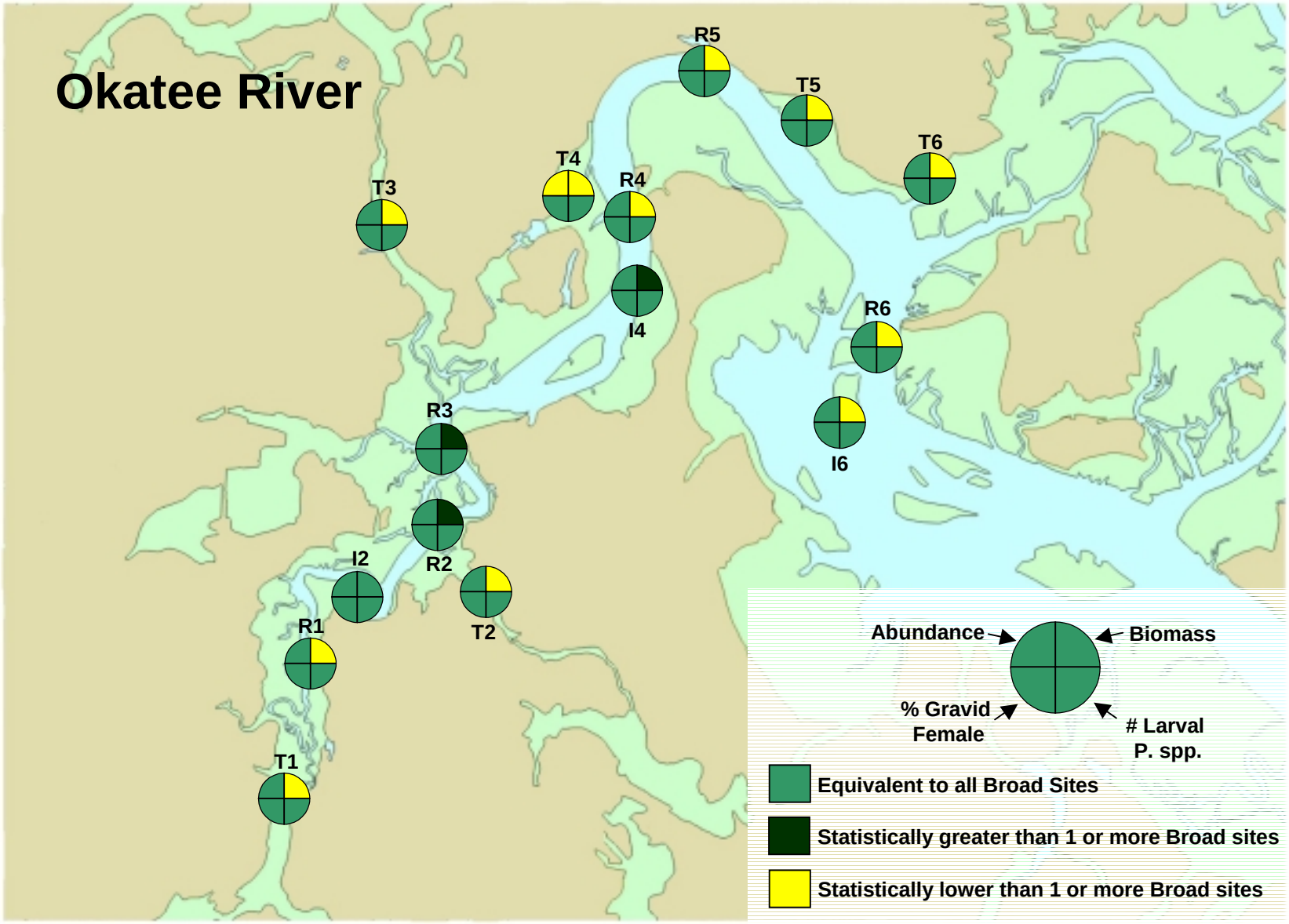


Figure 5.28. Map of Okatee River showing *Palaemonetes spp*. population metrics relative to Broad Creek.

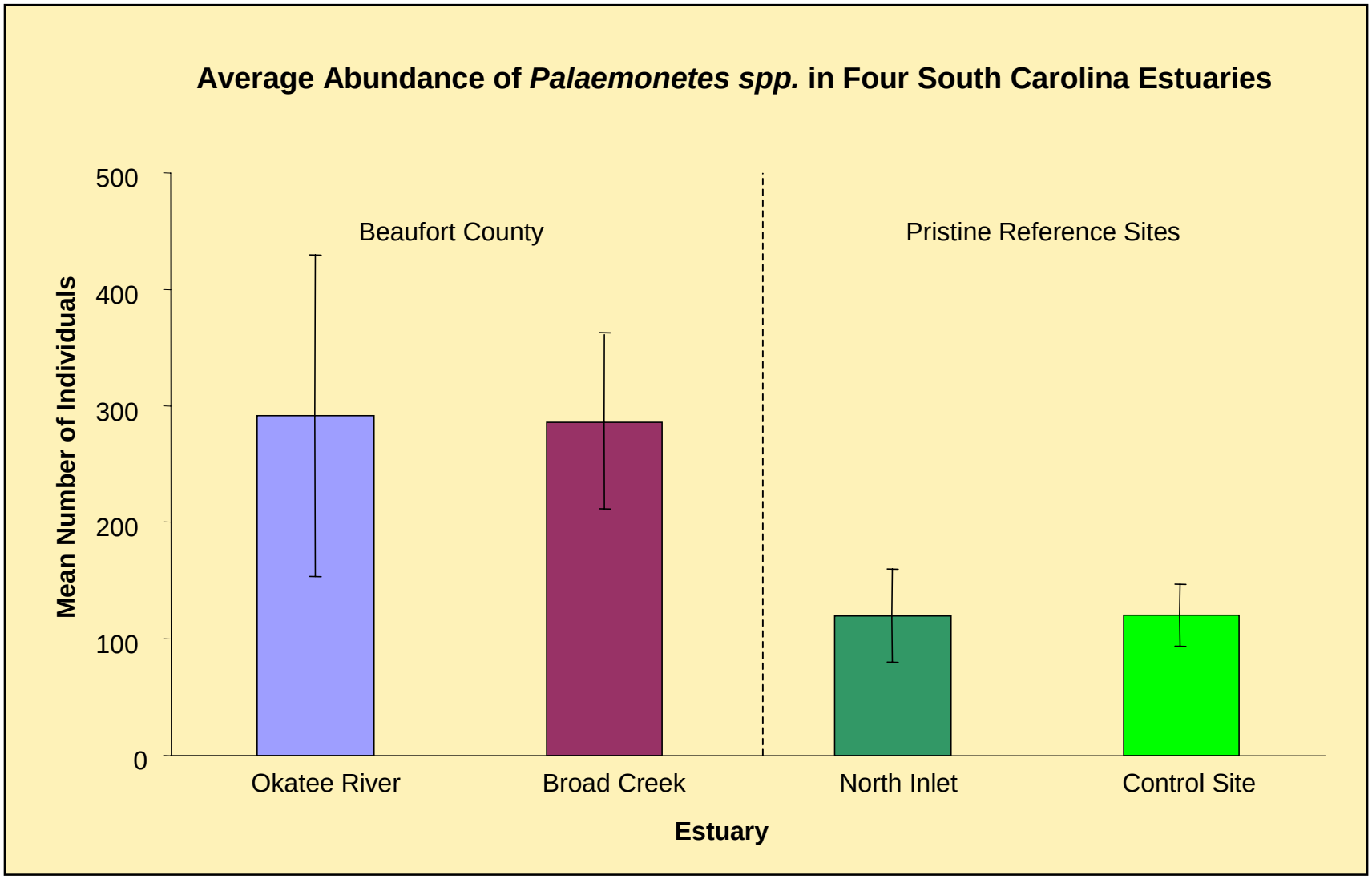


Figure 5.29. Comparison of *Palaemonetes pugio* abundance in Broad Creek and the Okatee River with relatively pristine sites in North Inlet and Leadenwah Creek.

Table 5.1. Ranked abundance of species comprising one percent or greater of the total subtidal community abundance from each system. Figures represent mean number/0.04 m^2. (A=amphipod; P=polychaete; O=oligochaete; M=mollusk; C=other crustacean; T=other taxa)

Species	Taxon code	Broad Creek Stations						Okatee River Stations					
		1	2	3	4	5	6	1	2	3	4	5	6
Ampelisca vadorum	A	0	0	0	0	0	0	0	0	0	7	1291	0
Streblospio benedicti	P	56	46	45	39	0	86	8	23	50	16	2	3
Parapionosyllis sp.	P	0	0	0	0	0	33	0	0	1	5	0	246
Tubificoides wasselli	O	0	0	0	0	0	13	0	17	220	16	1	5
Exogone dispar	P	0	0	0	0	0	0	0	0	1	202	41	0
Scoletoma tenuis	P	2	22	29	0	0	17	9	23	22	72	26	1
Tubificidae	O	54	0	4	11	0	32	0	1	0	7	37	10
Cirrophorus sp.	P	0	0	0	1	0	0	9	37	73	20	0	3
Mediomastus sp.	P	0	2	0	0	0	17	0	4	4	51	45	0
Streptosyllis sp.	P	0	0	0	59	0	1	0	0	5	1	0	44
Actiniaria	T	1	0	1	2	0	1	0	6	16	4	70	1
Enchytraeidae	O	0	0	0	0	0	5	0	0	0	0	0	69
Scoloplos rubra	P	1	14	19	0	0	0	1	3	5	17	3	0
Caulleriella sp.	P	0	0	0	0	0	13	0	0	7	28	0	5
Leptognatha caeca	T	0	0	0	46	0	0	0	0	0	0	0	0
Ilyanassa obsoleta	M	38	1	0	0	0	0	3	0	0	1	0	0
Heteromastus filiformis	P	19	12	1	0	0	2	3	0	0	0	1	0
Tubificoides brownae	O	10	1	3	0	0	2	6	5	2	1	6	3
Brania sp.	P	0	0	0	10	0	8	0	0	6	0	2	6
Monticellina sp.	P	0	0	0	0	0	16	0	1	0	9	2	0
Polydora sp.	P	19	3	2	0	0	1	0	0	0	0	0	0
Spiochaetopterus costarum	P	0	14	6	0	0	1	0	2	1	0	0	0
Cyathura burbancki	T	0	0	0	1	0	10	0	1	0	6	3	0
Protohaustorius bousfieldi	A	0	0	0	0	17	0	0	0	0	0	0	0
% of total abundance		95.1	89.1	81.2	78.2	81.3	77.0	77.7	85.1	89.7	81.1	95.4	93.4
mean total abundance		212.3	128.3	135.0	215.7	21.3	337.7	52.3	143.0	460.3	570.7	1602.7	426.3
mean # of species		13.3	10.3	19.7	16.0	2.3	39.0	11.3	18.0	26.0	42.0	33.0	18.7
mean H'		2.5	2.5	3.0	2.8	0.8	4.0	3.0	3.2	2.6	3.7	1.4	2.3
mean evenness		0.7	0.7	0.7	0.7	0.5	0.8	0.9	0.8	0.6	0.7	0.3	0.6

Table 5.2. Overall ranked abundance of species comprising one percent or greater of the total intertidal community abundance from each system. Values represent mean number/0.04 m^2. (A=amphipod; P=polychaete; O=oligochaete; M=mollusk; C=other crustacean; T=other taxa)

Species	Taxon code	Broad Creek			Okatee River		
		1	4	6	2	4	6
Streblospio benedicti	P	4	9	28	29	2	8
Monopylephorus rubroniveus	O	0	59	9	1	0	0
Scoletoma tenuis	P	0	0	4	29	12	12
Nereis succinea	P	1	0	0	22	11	0
Ilyanassa obsoleta	M	11	7	10	0	0	0
Leitoscoloplos fragilis	P	0	0	0	6	10	5
Brania sp.	P	16	0	0	0	0	0
Heteromastus filiformis	P	1	8	1	0	0	2
Tubificoides brownae	O	3	0	3	0	0	0
Nemertinea	T	2	1	0	1	2	1
Uca pugilator	C	0	0	0	5	0	0
Gastropoda	M	0	0	0	0	4	0
Decapoda	C	1	0	0	2	0	0
Aphealochaeta sp.	P	0	0	0	0	0	3
Eobrolgus spinosus	A	0	0	0	0	0	3
Tubificidae	O	0	0	0	0	0	3
Leitoscoloplos sp.	P	0	0	0	1	1	0
% of total abundance		92.0	99.2	98.8	97.3	95.5	84.1
mean total abundance		41.7	85.0	55.0	98.7	44.7	44.0
mean # of species		5.7	6.7	4.7	8.3	8.0	11.0
mean H'		1.5	1.5	1.7	1.7	2.3	2.8
mean evenness		0.6	0.6	0.8	0.6	0.8	0.8

Table 5.3. Overall ranked abundance of tidal creeks species. (A=amphipod; P=polychaete; O=oligochaete; M=mollusk; I=isopod; In=insect; T=tanaid)

Species	Taxon code	Broad Creek				Okatee River			
		Average No./m^2	Percent Total	Percent Occurrence	Rank	Average No./m^2	Percent Total	Percent Occurrence	Rank
Monopylephorus rubroniveus	O	2485.4	58.1	65	1	2072.4	44.9	55	1
Streblospio benedicti	P	625	14.6	60	2	201	4.4	25	6
Heteromastus filiformis	P	314.3	7.3	46.7	3	47.5	1	10	11
Tubificidae	O	168.1	3.9	26.7	4	522.7	11.3	43.3	2
Capitomastus aciculatus	P	160.8	3.8	25	5	14.6	0.3	3.3	16
Tubificoides brownae	O	106	2.5	20	6	142.5	3.1	13.3	7
Capitella capitata	P	98.7	2.3	16.7	7	36.6	0.8	10	14
Nereis succinea	P	91.4	2.1	15	8	478.8	10.4	46.7	3
Fabricia sp.	P	58.5	1.4	13.3	9	65.8	1.4	6.7	9
Monopylephorus irroratus	O	54.8	1.3	11.7	10	40.2	0.9	5	12
Eteone heteropoda	P	14.6	0.3	5	11				
Aphealochaeta sp.	P	11	0.3	5	12.5				
Nemertinea	N	11	0.3	5	12.5	54.8	1.2	20	10
Edotea montosa	I	7.3	0.2	3.3	17	3.7	0.1	1.7	21
Glycera americana	P	7.3	0.2	3.3	17				
Laeonereis culveri	P	7.3	0.2	3.3	17	3.7	0.1	1.7	21
Leitoscoloplos sp.	P	7.3	0.2	3.3	17	40.2	0.9	5	13
Marionina spartinae	O	7.3	0.2	3.3	17	7.3	0.2	1.7	17
Polydora cornuta	P	7.3	0.2	3.3	17	3.7	0.1	1.7	21
Sabellidae	P	7.3	0.2	3.3	17				
Ampharetidae	P	3.7	0.1	1.7	24.5				
Corbiculidae	M	3.7	0.1	1.7	24.5	3.7	0.1	1.7	21
Drilonereis longa	P	3.7	0.1	1.7	24.5				
Eulalia sanguinea	P	3.7	0.1	1.7	24.5				
Exogone dispar	P	3.7	0.1	1.7	24.5				
Mediomastus californiensis	P	3.7	0.1	1.7	24.5				
Mercenaria mercenaria	M	3.7	0.1	1.7	24.5				
Sphaerosyllis longicauda	P	3.7	0.1	1.7	24.5				
Tubificoides heterochaetus	O					460.5	10	13.3	4
Colembolla	In					255.9	5.5	3.3	5
Cyathura polita	I					127.9	2.8	18.3	8
Tubificoides wasselli	O					18.3	0.4	3.3	15
Hesionidae	P					3.7	0.1	1.7	21
Leptochelia rapax	T					3.7	0.1	1.7	21
Rhepoxynius epistomus	A					3.7	0.1	1.7	21

Table 5.4. Summary of tidal creek environment quality measures. Values in shaded areas are within ranges that indicate degraded conditions.

Broad Creek

Stress Measure	Tidal Creek Stations						
	T1	T2	T3	T4	T5	T6	Mean
Salinity range	**22.3**	2	10.3	13.6	1.5	**27.1**	12.8
Silt/clay content	34	34	19	30	50	74	40.2
% of time < 2mg/l	18	0	0	3	**20**	0	6.8
ERMQ	**0.078**	0.023	0.013	0.033	0.030	0.011	0.031
% of fauna as oligochaetes	**96**	36	17	39	**80**	13	46.8
% of *Monopylephorus rubroniveus*	**90**	30	12	19	**78**	13	40.3
Species diversity (H')	**0.23**	0.60	0.85	0.96	**0.39**	0.76	1
Benthic abundance (#/m^2)	6,619	3,143	921	5,657	7,983	1,009	4,222
Overall assessment	**Degraded**	Normal	Normal	Normal	**Degraded**	Normal	Normal

Okatee River

Stress Measure	Tidal Creek Stations						
	T1	T2	T3	T4	T5	T6	Mean
Salinity range	**26.4**	3.1	1.2	**18.2**	1	**22.5**	12.1
Silt/clay content	46	41	23	88	6		40.8
% of time < 2mg/l	0	2	0	0	0	0	0.3
ERMQ	0.024	0.025	0.017	**0.049**	0.031	**0.050**	0.032
% of fauna as oligochaetes	**99**	67	**70**	25	50.4	20.9	55.38
% of *Monopylephorus rubroniveus*	**75**	10	18	17	33	10	27.17
Species diversity (H')	**0.31**	0.67	0.82	0.62	0.89	0.66	0.7
Benthic abundance (#/m^2)	12,698	2,719	2,281	1,733	5,000	3,246	4612.8
Overall assessment	**Degraded**	Normal	Normal	Normal	Normal	Normal	Normal

Table 5.5. Contaminant analytes measured in oyster tissue

Metals
Aluminum
Arsenic
Cadmium
Chromium
Copper
Lead
Manganese
Mercury
Nickel
Silver
Tin
Zinc

PAHs
2-Methyl Naphthalene
Acenapthene
Acenapthylene
Anthracene
Benzo(A) Anthracene
Benzo(A) Pyrene
Benzo(B) Fluoranthene
Benzo(Ghi) Perylene
Benzo(K) Fluoranthene
Chrysene
Dibenzo(A,H) Anthracene
Fluoranthene
Fluorene
Naphthalene
Phenanthrene
Pyrene

PCBs
PCB-1016
PCB-1221
PCB-1232
PCB-1242
PCB-1248
PCB-1254
PCB-1260

Pesticides
Aldrin
Alpha Endosulfan
Alpha-BHC
Beta Endosulfan
Beta-BHC
Chlordane
Delta-BHC
Diazinon
Dieldrin
Dursban (Chlorpyrifos)
Endosulfan Sulfate
Endrin
Endrin Aldehyde
Heptachlor
Heptachlor Epoxide
Lindane
P,P'DDD
P,P'DDE
P,P'DDT
Toxaphene

Biological
Percent Fat

Table 5.6 Tissue contaminant concentrations in oyster samples collected from sites in Broad Creek and the Okatee River.

River	Station	Date	Copper Tissue mg/kg Wet Wt	Copper mg/kg Calc. Dry Wt	Zinc Tissue mg/kg Wet Wt	Zinc mg/kg Calc. Dry Wt	Cadmium Tissue mg/kg Wet Wt		Cadmium mg/kg Calc. Dry Wt
Broad	O-1	97/8/26	16	159.8	370	3694.8	0.2	K	2.0
Broad	O-2	97/8/26	23	229.7	340	3395.2	0.2	K	2.0
Broad	O-3	97/8/26	27	269.6	350	3495.1	0.2		2.0
Broad	O-4	97/8/27	16	159.8	270	2696.2	0.2		2.0
Broad	O-5	97/8/27	15	149.8	280	2796.1	0.2		2.0
Broad	O-6	97/8/27	6.5	64.9	140	1398.0	0.3		3.0
Okatee	O-1	97/9/10	10	99.9	**660**	**6590.8**	0.4		4.0
Okatee	O-2	97/9/10	12	119.8	460	4593.6	0.3		3.0
Okatee	O-3	97/9/10	12	119.8	400	3994.4	0.4		4.0
Okatee	O-4	97/9/11	12	119.8	380	3794.7	0.4		4.0
Okatee	O-5	97/9/11	7.4	73.9	230	2296.8	0.2		2.0
Okatee	O-6	97/9/11	16	159.8	410	4094.3	0.5		5.0

Exceeds mean of annual geometric means reported by O'Connor (1996) for oysters collected from US coastal stations from 1986 - 1993.

Exceeds 90th percentile of wet weight concentration collected from South Carolina by SCDHEC from 1980 - 1997.

K = Below Limit of Detection

Table 5.7. Summary of changes in the areal extent of shellfish beds sampled in Broad Creek and the Okatee River in 1984-85 versus 1997.

	Square Feet			Total Volume (bushels)			Live Volume (bushels)		
Broad Creek	**1984**	**1997**	**% Change**	**1984**	**1997**	**% Change**	**1984**	**1997**	**% Change**
O-1	3,300	6,382	93	359	612	70	148	231	56
O-2	13,200	13,425	2	1,856	1,476	-20	790	548	-31
O-3	3,960	5,755	45	909	538	-41	366	158	-57
O-4	3,600	7,006	95	826	1,114	35	332	396	19
O-5	6,416	5,492	-14	1,327	473	-64	543	68	-87
O-6	3,300	2,730	-17	757	1,181	56	305	666	118
Okatee River	**1985**	**1997**	**% Change**	**1985**	**1997**	**% Change**	**1985**	**1997**	**% Change**
O-1	0*	1,764	NA	0*	174	NA	0*	72	NA
O-2	7,200	10,140	41	860	863	0	321	356	11
O-3	16,440	11,523	-30	1,869	768	-59	736	317	-57
O-4	8,664	4,420	-49	1,060	334	-68	386	138	-64
O-5	3,300	4,373	33	443	272	-39	146	115	-21
O-6	11,050	8,030	-27	1,311	608	-54	493	225	-54

* 1985 survey indicated no intertidal shellfish bed at this site

All data provided by the SCDNR Office of Fisheries Management, Shellfish Management Section.

Table 5.8. Statistical comparisons of Broad Creek and Okatee River grass shrimp samples (a = 0.05 for all tests). Shading indicates significant differences. An asterisk indicates that the test for normality passed (with p > 0.05), but the test for equal variance failed, and a nonparametric test was used for that comparison.

Comparisons	Parameter	Normality	(p value)	Test	Result	(p value)	Multiple Comparison Test	Pairwise Comparisons
System-wide	Abundance	failed	<0.0001	Mann-Wh.	sig. diff.	0.0319		Broad > Okatee
(Okatee vs. Broad)	Biomass	failed	<0.0001	Mann-Wh.	sig. diff.	0.029		Broad > Okatee
	% Gravid Female	failed	<0.0001	Mann-Wh.	no sig. diff.	0.287		
	Larval	failed	<0.0001	Mann-Wh.	no sig. diff.	0.799		
	Average Size	failed	0.0078	Mann-Wh.	no sig. diff.	0.56		
Between Habitats	Abundance	failed	<0.0001	Kruskal-Wallis	no sig. diff.	0.823		
(within Okatee)	Biomass	failed	0.0036	Kruskal-Wallis	no sig. diff.	0.831		
	% Gravid Female	failed	0.029	Kruskal-Wallis	no sig. diff.	0.359		
	Larval	failed	<0.0001	Kruskal-Wallis	no sig. diff.	0.235		
	Average Size	failed	0.0134	Kruskal-Wallis	no sig. diff.	0.217		
Between Habitats	Abundance	failed	<0.0001	Kruskal-Wallis	no sig. diff.	0.27		
(within Broad)	Biomass	passed	0.0541	1 way ANOVA	no sig. diff.	0.282		
	% Gravid Female	passed	0.0611	1 way ANOVA	no sig. diff.	0.542		
	Larval	failed	0.0205	Kruskal-Wallis	sig. diff.	0.0084	Dunn's	River > Tidal
	Average Size	passed	0.2106	1 way ANOVA	no sig. diff.	0.631		
Tidal Data	Abundance	failed	0.0022	Mann-Wh.	no sig. diff.	0.174		
(Okatee vs Broad)	Biomass	* passed	0.1883	Mann-Wh.	no sig. diff.	0.137		
	% Gravid Female	failed	0.0106	Mann-Wh.	no sig. diff.	0.216		
	Larval	failed	<0.0001	Mann-Wh.	no sig. diff.	0.428		
	Average Size	failed	0.0013	Mann-Wh.	no sig. diff.	1		
River Data	Abundance	failed	<0.0001	Mann-Wh.	no sig. diff.	0.373		
(Okatee vs Broad)	Biomass	failed	0.001	Mann-Wh.	no sig. diff.	0.468		
	% Gravid Female	failed	0.0023	Mann-Wh.	sig. diff.	0.035		Broad > Okatee
	Larval	failed	<0.0001	Mann-Wh.	no sig. diff.	0.2		
	Average Size	* passed	0.385	Mann-Wh.	no sig. diff.	0.718		
Intertidal Data	Abundance	failed	0.0486	Mann-Wh.	no sig. diff.	0.4799		
(Okatee vs Broad)	Biomass	passed	0.3483	t-test	no sig. diff.	0.4083		
	% Gravid Female	passed	0.1209	t-test	no sig. diff.	0.683		
	Larval	passed	0.2446	t-test	no sig. diff.	0.1792		
	Average Size	passed	0.084	t-test	no sig. diff.	0.7439		

Table 5.9. Summary of population metrics collected for mummichog (*Fundulus heteroclitus*) populations

		Broad Creek					Okatee River					
Metric	Station	T-1	T-2	T-3	T-4	T-5	T-1	T-2	T-3	T-4	T-5	T-6
Condition Index	avg	2.46	2.41	2.38	2.32	2.58	2.36	2.27	2.30	2.28	2.35	2.39
	std	0.27	0.20	0.22	0.24	0.21	0.24	0.15	0.17	0.17	0.31	0.21
Total Length	avg	59.43	52.55	51.52	62.87	66.01	49.96	47.09	45.92	43.27	57.32	44.56
	std	12.88	11.66	11.63	12.96	13.09	8.55	4.47	6.46	6.87	13.81	6.18
Standard Length	avg	48.55	42.87	41.97	51.02	54.23	41.06	38.00	36.98	34.86	46.09	36.17
	std	10.60	9.64	9.62	10.93	10.95	7.63	3.73	5.26	5.80	11.16	5.02
Weight	avg	3.28	2.25	2.14	3.60	4.71	1.82	1.28	1.25	1.05	2.84	1.21
	std	2.27	1.72	2.02	2.49	2.97	1.19	0.37	0.75	0.46	2.89	0.50
Abnormalities	% abnormal	0.78	0.00	0.71	4.46	1.08	3.92	0.00	3.37	0.00	6.38	0.00
Sex Ratio	M : F	49:77	99:129	118:163	50:60	96:90	10:41	23:29	39:135	14:26	18:29	22:31
Sample Size	m	49	99.0	118	50	96	10	23	39	14	18	22
	f	77	129.0	163	60	90	41	29	135	26	29	31
	I	2	1.0	0	2	0	0	4	4	4	0	1
	total	128	229	281	112	186	51	56	178	44	47	54
	% female	60.2	56.3	58.0	53.6	48.4	80.4	51.8	75.8	59.1	61.7	57.4